VOLUME LXVII NUMBER 5

THE

BOTANICAL GAZETTE

MAY 1919

EFFECT OF ANESTHETICS UPON RESPIRATION

A. R. C. HAAS

(WITH SEVEN FIGURES)

The special interest which this subject has acquired, as the result of certain modern theories, makes it desirable to give a brief review of some of the more important contributions (**5, 10**) to our knowledge of it.

MEYER (**19**) and OVERTON (**21**) independently concluded that the effect of a narcotic increases with its solubility in substances of a lipoid nature. According to them narcosis does not appear until the lipoids of the cells have absorbed the narcotic to a definite molecular concentration (**21**). The theory has been criticized (**5, 10**) because it fails to explain why narcotics, such as benzamide and monacetin, which at higher temperature are less soluble in fat, have an effect which increases with the temperature. Both MEYER and OVERTON recognize the fact that often there is no relation between the narcotic power of a substance and its relative solubility in oil; the partition quotient for isobutyl alcohol is about 180 times greater than for ethyl alcohol, but its power to cause narcosis is only about 6 times as great as that of ethyl alcohol. They state that such cases cannot be used as arguments against the lipoid theory in case the narcotic is not chemically indifferent, but has a special reaction affinity, as is the case, for example, with the basic narcotics.

VERWORN (**29**) has advocated the view that narcotics interfere with the oxygen carriers of the cell and render them incapable of

activating the molecular oxygen. As a result oxidation cannot take place and disintegration occurs, the cells thereby being asphyxiated. In this connection he makes the statement that a more or less complete recovery from narcosis, which may occur even in an oxygen-free medium, is at the cost of the oxygen contained within the living substance, which (on account of the suppression of the oxidation processes) could not be consumed.

MANSFELD'S (16) view is not essentially different from that of VERWORN. He believes that because the lipoids take up the narcotic, their power to absorb oxygen is decreased. Narcotized cells cannot take up sufficient oxygen for their needs and hence irritability is decreased by lack of oxygen. That narcotics do decrease the ability of olive oil to dissolve oxygen, has been asserted by HAMBURGER (8), although objection to his experiments has been made by WINTERSTEIN (31). In many cases it is certain that narcosis has nothing to do with absorption of oxygen. Thus WINTERSTEIN has observed that on anesthetizing the anaerobic worm *Ascaris* in absence of oxygen, it comes to rest very quickly under the influence of the anesthetic.

Experiments by numerous investigators have shown that narcosis and decrease of oxidation are not parallel. WARBURG (30) has observed that some narcotics decrease the oxidation of the erythrocytes of geese as much as 30–70 per cent. He has also observed, however, that narcotics do not always decrease the consumption of oxygen, for he has found that the segmentation of fertilized sea urchin eggs can be inhibited by phenylurethane without a perceptible decrease of the consumption of oxygen. LOEB and WASTENEYS (14) have obtained similar results with chloral hydrate, chloroform, and alcohol. NOTHMANN-ZUCKERKANDL (20) observed that the protoplasmic streaming of plant cells is quickly brought to a standstill by narcotics. Such cells have been shown to be rather insensitive to lack of oxygen, in that the streaming is stopped in the absence of oxygen only after several weeks.

BÜRKER (4) was of the opinion that because of the great solubility of narcotics in the lipoid of the cells, there is a competition between the lipoids and other substances in the protoplasm for the active oxygen, such that the cells are more or less in a state of

asphyxiation. His hypothesis maintains that the absorption of oxygen is not reduced during narcosis, but that the oxygen is prevented from going to its usual point of attack. This would necessitate the assumption that oxygen is more readily absorbed by the lipoids than by the other substances of the cell, which is not the case. An objection to all theories which make narcosis conditional on lipoid solubility is the fact that magnesium sulphate and carbon dioxide, which are not soluble in lipoid, produce typical anesthesia.

HÖBER (10) has formulated the hypothesis that narcosis is due to inhibition of enzymatic processes, brought about by a decrease in dispersion. This hypothesis is favored by the investigations of BATELLI and STERN (2), who found that proteins, such as the nucleo-proteins, are influenced by narcotics in approximately the same relative and absolute concentrations as those at which the enzymes are affected. In this connection VERNON (28) found that most narcotics are harmless to oxidases up to a definite limiting concentration, beyond which injury occurs. HÖBER explains the retardation or inhibition of enzyme action as due to the fact that narcotics go into the surface between the enzymes and the medium in which they are dispersed, thereby displacing the substratum on which the enzymes act.

The investigations hitherto discussed are largely based on measurements of the absorption of oxygen. In many cases such measurements could be made more accurately than determinations of the amount of carbon dioxide produced. Since the writer has recently been able to develop a method for the measurement of minute amounts of carbon dioxide in solution, it seemed to him that a fresh investigation of the subject by means of this method was desirable.

Previous investigations on the effect of narcotics on the production of carbon dioxide have yielded somewhat contradictory results. APPLEMAN (1) found that vapor of ethyl bromide approximately doubles the respiration of potatoes. MAYER (18) found that 0.25 per cent prussic acid stops the respiration of higher plants entirely. SCHROEDER (24) observed a decrease in respiration when *Aspergillus* was treated with ether. He found that prussic acid

inhibited the production of CO_2, while the absorption of oxygen continued. KOSINSKI (12) found that low concentrations of ether increased the respiration of *Aspergillus*, while higher concentrations decreased it. LAURÉN (13) and also IRVING (11) have noted that respiration increases during anesthesia produced by ether and chloroform. TASHIRO (26) found that anesthetics greatly reduce the output of CO_2 by dry seeds. BONNIER and MANGIN (3), as the result of experimental work upon the influence of measured quantities of ether upon flowering plants, concluded that the respiratory activity is unaffected by anesthetics. It has since been shown by EWART (6) that chloroform increases the respiratory activity in *Elodea*.

The effect of anesthetics upon the respiration of marine plants has received very little attention. HARDER (9) has made determinations of the respiration of marine algae, but in no case has he studied the effect of anesthetics. PANTANELLI (22) has observed that sea water, when half saturated with chloroform, reduces the excretion of CO_2 to about one-half of the normal. His experiments were very few, and no duplicate or control experiments were made. The methods employed by PANTANELLI and HARDER often required that plants be shut up air-tight, in flasks completely filled with sea water, and left in this condition for several hours, analyses being made at the beginning and end of these long periods.

In the experiments of the writer on the effect of anesthetics upon the production of CO_2, the marine alga *Laminaria* was found to be well suited to the purpose. Fronds were cut up into pieces about 2 inches long. Each piece was rolled up loosely and inserted into a piece of Pyrex glass tubing. This was closed at one end, while a piece of paraffined rubber tubing was attached to the open end. Sea water, of approximately the same temperature as the material, was then added to the tubes and the rubber tube closed by a spring clamp. The tubes were then brought very gradually to the temperature (16° C.) of the constant temperature bath. The tubes were kept dark by inserting each tube in a black-enameled, collapsible tin tube submerged in the bath. Several tubes were used as controls in each experiment. These contained material in sea water without any addition. The reagents were

always used at the same temperature as that of the water bath. After the tubes containing the *Laminaria* in sea water had been at 16° C. from half an hour to an hour, the solution was poured out of each tube and replaced by fresh sea water at 16° C. This was repeated several times before beginning an experiment.

After a tube containing *Laminaria* in sea water had been clamped off so as to include a small bubble of air which could be used as a stirrer, and had been exposed to 16° C. for a definite period, it was removed from the bath. The solution was slightly stirred by inverting the tube a few times; the clamp was then opened and the solution rapidly poured into another empty tube to which the same number of drops of indicator (phenolsulphonephthalein) had been added as was added to the buffer solutions (7). In order to mix the solution with the indicator it was stirred as described, and was then compared with buffer solutions of a known PH value which contained the same amount of indicator.

The use of a small bubble of air as a stirring agent was found to be very convenient, and when compared with the use of paraffined glass globules as stirrers was found to introduce no error of any importance.

The buffer solutions (27) were made up by mixing M/15 Na_2HPO_4 and M/15 KH_2PO_4 in various proportions. They were of the same diameter as those containing the alga. A 0.01 per cent aqueous solution of phenolsulphonephthalein served as the indicator of the PH values. The indicator solution was used at the rate of 5 drops in 10 cc. of solution. A correction (30) of 0.30 for the salt error of the indicator in sea water was subtracted from the observed readings of the PH value of sea water. The use of a constant source of light (the "Daylight" lamp) permitted observations to be made in a uniform manner. The decrease in PH, which results from the production of CO_2, served as a criterion of the amount of respiration.

In all the experiments, each tube was observed for a number of periods (always of the same length) for each piece of material, until it was evident that the rate of respiration had become practically constant. Several of the tubes were then used as controls, while to the others was added the sea water containing the

anesthetic. In every case tubes which contained only sea water showed that the apparatus was not responsible for any of the changes observed in the experiments in which tissue was used.

The solutions used in any one experiment were always of the same PH value, so that the results were comparable. Many of the anesthetics, especially when in small concentrations, do not appreciably affect the PH value of the sea water. A large number of carboys of sea water, obtained from Woods Hole at the same time, were filtered to remove organic matter in varying degrees, the unfiltered material settling to the bottom. When an anesthetic decreased the PH value only slightly, it was possible by a suitable selection of the sea water from the carboys to obtain a bottle of sea water of a PH value equal to that of the sea water containing the anesthetic.

Whenever large amounts of an anesthetic were used in the sea water, it was considered advisable to add concentrated sea water to bring the electrical conductivity of the solution up to that of sea water. This was done with each of the following solutions: sea water containing 16.1 and 24.2 per cent alcohol respectively; sea water containing 17.4 per cent acetone; and sea water containing 3.2 per cent formaldehyde. Many of the experiments were repeated at Woods Hole in the summer of 1917. In these experiments the solutions containing alcohol (all above 1 per cent) were made of the same electrical conductivity as sea water. The results were practically the same as when no concentrated sea water was added.

In the case of the higher concentration of formaldehyde, the free acid was first neutralized with sodium carbonate. This is allowable for the purposes of the present investigation, as its only effect would be to make the amount of CO_2 produced appear somewhat less than was actually the case. It should be understood that the percentages of liquid anesthetics in the following descriptions are percentages by volume. In an experiment in which there is considerable dilution, as when absolute alcohol is added to sea water to make a 10 per cent alcoholic solution, it might be conceivable that the dilution might be the cause of the increased rate of respiration. For that reason experiments were made with

pieces of material in normal sea water until constant values for the respiration were obtained; then sea water diluted with 10 per cent of tap water was used, and it was found that the dilution produced no appreciable effect.

In each experiment 6 cc. of solution was used. At the end of each period, after noting the new PH value, 6 cc. of fresh solution was poured into the tube, which was then treated as before. It was at first thought that in pouring out the solution from the tube containing the tissue sufficient might be held back by the tissue to affect the observed PH value. Actual determinations proved that this was not the case.

In view of the fact that there may be an increase of respiration as the result of injury (**23**), preliminary experiments were made to ascertain whether the cutting of the *Laminaria* had any appreciable effect upon the respiration. It was found that the change in the respiration due to cutting was negligible (the cutting was always reduced to a minimum).

In some of the experiments there was noted a very slight decrease in the production of CO_2 as time went on, although not enough to be of significance for the present investigation. This phenomenon has been observed by Miss MATTHAEI (**17**) in determinations of CO_2 in connection with experiments on photosynthesis. Such a decrease in respiration has been attributed by her to the gradual decrease of substances available for oxidation.

The results of the experiments are given both in tables and figures. In each experiment 6–12 (or more) closely agreeing determinations were obtained and the results of a typical case were taken. The plant was always placed for a definite time in sea water or sea water containing the reagent, and at the end of this time the PH value was determined. This interval is in every case shown in the tables as well as by the points on the curves. At the end of each interval the solution was renewed.

The alteration of the PH value is an index of the amount of CO_2 produced, since the greater the amount of CO_2 the greater the decrease in PH. Since this relation is approximately linear in this range of PH values, it is not necessary to translate the decrease in PH value into cc. of CO_2 produced, as the form of the respiration

curve would be practically the same whether we use as ordinates PH values or cc. of CO_2 produced. The curves are plotted in such a manner that the abscissae represent time (in minutes) and the ordinates represent the change in PH value corresponding to either the relative amount of respiration, the relative rate of respiration, or to both (as designated in the figures). The relative amount of respiration is obtained by dividing the total change in PH during exposure to the reagent by the total change in PH caused by the

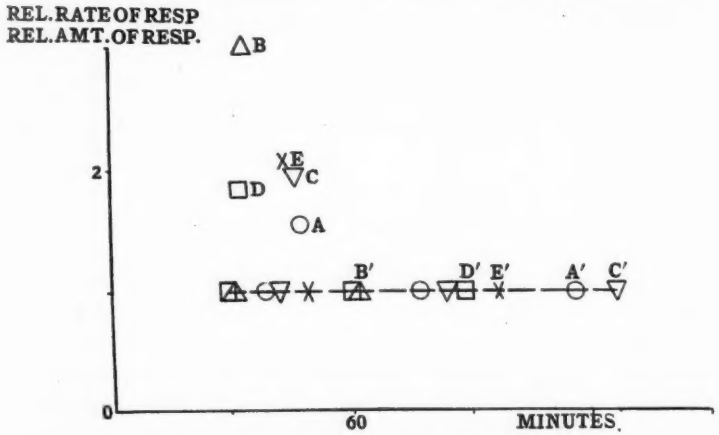


FIG. 1*a*.—Points showing relative rate and relative amount (identical in this case) of respiration of *Laminaria* produced by sea water containing *A*, 0.1 per cent chloral hydrate; *B*, 0.1 per cent novocain; *C*, 1 per cent ether; *D*, 0.1 per cent caffeine; *E*, ethyl-bromide; controls in sea water (broken lines); see tables I A to E; each control bears the same letter (with a prime) as the experimental curve.

same material under normal conditions during the same length of time. The relative rate is obtained by dividing the change in PH during one period by the change produced during a similar period by the same material under normal conditions. The broken lines in each case represent controls in sea water. The curve of each control bears the same symbol and letter as the experimental curve for sea water plus anesthetic, except that in the control the letters are primed.

Inspection of the results for 0.1 per cent chloral hydrate (fig. 1*a* [A]; table I A), 0.1 per cent novocain (fig. 1*a* [B]; table I B),

TABLE I A*

PERIOD OF 47.75 MIN. IN SEA WATER AND EQUAL PERIOD IN SEA WATER CONTAINING 0.1 PER CENT CHLORAL HYDRATE

Solution	Change in PH	Relative rate of respiration
Sea water	7.90−7.55=0.35	
Sea water containing 0.1 per cent chloral hydrate	7.90−7.35=0.55	0.55÷0.35=1.57

* In the control, with 3 periods (38.75 min. each) in sea water, the change in PH in each period was 7.90−7.55=0.35.

TABLE I B*

PERIOD OF 33.25 MIN. IN SEA WATER FOLLOWED BY EQUAL PERIOD IN SEA WATER CONTAINING 0.1 PER CENT NOVOCAIN

Solution	Change in PH	Relative rate of respiration
Sea water	7.65−7.40=0.25	
Sea water containing 0.1 per cent novocain	7.65−6.90=0.75	0.75÷0.25=3

* In the control, with 2 periods (30.75 min. each) in sea water, the change in PH in each period was 7.65−7.40=0.25.

TABLE I C*

PERIOD OF 45.25 MIN. IN SEA WATER AND EQUAL PERIOD IN SEA WATER CONTAINING 1 PER CENT (BY VOLUME) OF ETHER

Solution	Change in PH	Relative rate of respiration
Sea water	7.90−7.57=0.33	
Sea water containing 1 per cent ether	7.90−7.25=0.65	0.65÷0.33=1.97

* In the control, with 3 periods (42 min. each) in sea water, the change in PH in each period was 7.90−7.60=0.30.

TABLE I D*

TWO PERIODS (32.5 MIN. EACH) IN SEA WATER AND SAME LENGTH OF TIME IN SEA WATER CONTAINING 0.1 PER CENT CAFFEINE

Solution	Change in PH	Relative rate of respiration
Sea water	7.65−7.43=0.22	
" "	7.65−7.43=0.22	
Sea water containing 0.1 per cent caffeine	7.65−7.25=0.40	0.40÷0.22=1.8

* In the control, with 3 periods (29.5 min. each) in sea water, the change in PH in each period was 7.65−7.50=0.15.

TABLE I E*

PERIOD (43.25 MIN.) IN SEA WATER FOLLOWED BY EQUAL PERIOD IN SEA WATER APPROXIMATELY SATURATED WITH ETHYL BROMIDE

Solution	Change in PH	Relative rate of respiration
Sea water	7.90−7.35=0.55	
Sea water saturated with ethyl bromide	7.90−6.75=1.15	1.15÷0.55=2.1

* In the control, with 2 periods (49.25 min. each) in sea water, the change in PH in each period was 7.90−7.30=0.60.

TABLE I F

THREE PERIODS (22 MIN. EACH) IN SEA WATER AND 7 EQUAL PERIODS IN SEA WATER APPROXIMATELY SATURATED WITH ETHYL BROMIDE

Solution	Change in PH	Relative rate of respiration
Sea water	8.1 −7.8 =0.30	
" "	8.1 −7.8 =0.30	
Sea water containing ethyl bromide	7.32−6.16*=1.16+	1.16÷0.30=3.8
" " " " "	7.32−6.16*=1.16+	1.16÷0.30=3.8
" " " " "	7.32−6.69 =0.63	0.63÷0.30=2.1
" " " " "	7.32−7.07 =0.25	0.25÷0.30=0.8
" " " " "	7.32−7.21 =0.11	0.11÷0.30=0.4
" " " " "	7.32−7.32 =0.0	0.0 ÷0.30=0.0

* Approximate (at this point the indicator is not very sensitive to slight changes in acidity).

TABLE I F CONTROL

SEVEN PERIODS (27.5 MIN.) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1	8.1−7.6 =0.5	0.5 ÷0.5=1.00
2	8.1−7.6 =0.5	0.5 ÷0.5=1.00
3	8.1−7.6 =0.5	0.5 ÷0.5=1.00
4	8.1−7.6 =0.5	0.5 ÷0.5=1.00
5	8.1−7.65=0.45	0.45÷0.5=0.90
6	8.1−7.65=0.45	0.45÷0.5=0.90
7	8.1−7.65=0.45	0.45÷0.5=0.90

1 per cent ether (fig. 1*a* [C]; table I C), 0.1 per cent caffeine (fig. 1*a* [D]; table I D), and for sea water approximately saturated with ethyl bromide (fig. 1*a* [E]; table I E; fig. 1*b* [A and B]; table I F) shows that these anesthetics increase both the relative amount and relative rate of respiration.

In fig. 1*b* (A) it will be seen that in sea water containing ethyl bromide the relative rate of respiration is greatly increased during the first 2 periods and then drops to the normal rate in about 90 min. After 132 min. no excretion of CO_2 could be detected. When we plot the curve B (fig. 1*b*), in which the ordinates represent the relative amount of respiration, we find that the curve is far above the base line even when the respiration cannot be detected.

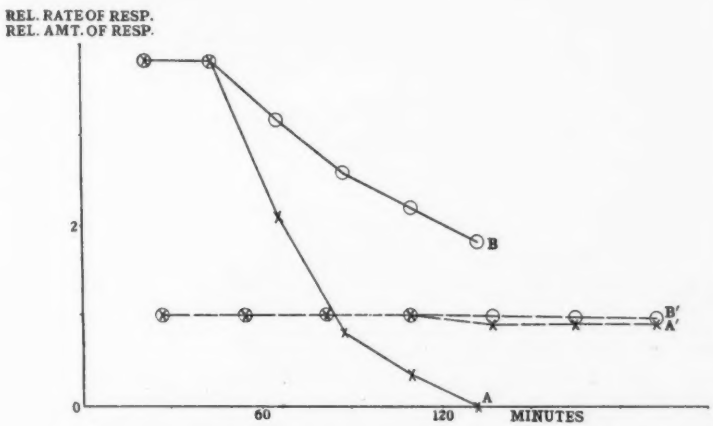


FIG. 1*b*.—Curves showing effect of sea water approximately saturated with ethyl bromide upon *A*, relative rate of respiration; B, relative amount of respiration of *Laminaria* (unbroken lines); controls in sea water (broken lines); see table I F; each control bears the same letter (with a prime) as the experimental curve.

The fact that ethyl bromide appears to have a marked accelerating effect upon the respiration confirms the results of APPLEMAN (1), who observed that when potato tubers are exposed to ethyl bromide vapor the respiration is greatly increased. Fig. 1*b* makes it evident that there is no initial decrease in the respiration.

The ethyl bromide referred to in table I F was acid, and since it was not neutralized by adding sodium carbonate it caused the PH of the sea water plus anesthetic to be lower than that of the sea water alone. In experiments with all other substances both sea water and sea water plus anesthetic were of the same PH value at the beginning of the experiment.

The respiration of *Laminaria* was followed for several hours, when the tissue was placed in solutions of sea water containing 3.2 per cent (fig. 2*a* [A], fig. 2*b* [A]; table II A) and 0.8 per cent

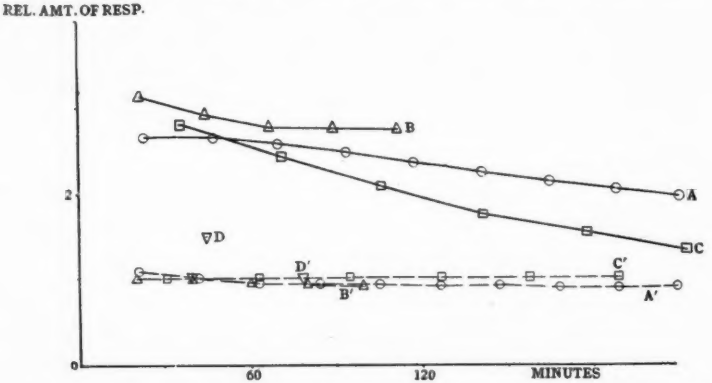


FIG. 2*a*.—Curves (unbroken lines) showing the effect upon the relative amount of respiration of *Laminaria* of sea water containing *A*, 3.2 per cent formaldehyde; *B*, 0.8 per cent formaldehyde; *C*, 0.3 per cent chloroform; *D*, 0.05 per cent chloroform; controls in sea water (broken lines); see tables II A to II D; each control bears the same letter (with a prime) as the experimental curve.

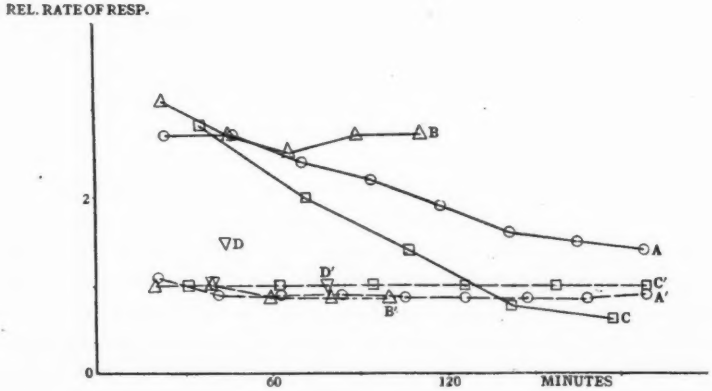


FIG. 2*b*.—Curves (unbroken lines) showing effect upon relative rate of respiration of *Laminaria* of sea water containing *A*, 3.2 per cent formaldehyde; *B*, 0.8 per cent formaldehyde; *C*, 0.3 per cent chloroform; *D*, 0.05 per cent chloroform; controls in sea water (broken lines); each control bears the same letter (with a prime) as the experimental curve.

TABLE II A

TWO PERIODS (23.5 MIN. EACH) IN SEA WATER AND 13 EQUAL PERIODS IN SEA WATER CONTAINING 3.2 PER CENT FORMALDEHYDE (8 PER CENT BY VOLUME OF 40 PER CENT FORMALDEHYDE)

Solution	Change in PH	Relative rate of respiration
Sea water	8.37−7.82=0.55	
" "	8.37−7.82=0.55	
Sea water containing 3.2 per cent formaldehyde	8.37−6.90=1.47	1.47÷0.55=2.7
Sea water containing 3.2 per cent formaldehyde	8.37−6.90=1.47	1.47÷0.55=2.7
Sea water containing 3.2 per cent formaldehyde	8.37−7.05=1.32	1.32÷0.55=2.4
Sea water containing 3.2 per cent formaldehyde	8.37−7.15=1.22	1.22÷0.55=2.2
Sea water containing 3.2 per cent formaldehyde	8.37−7.33=1.04	1.04÷0.55=1.9
Sea water containing 3.2 per cent formaldehyde	8.37−7.50=0.87	0.87÷0.55=1.6
Sea water containing 3.2 per cent formaldehyde	8.37−7.55=0.82	0.82÷0.55=1.5
Sea water containing 3.2 per cent formaldehyde	8.37−7.58=0.79	0.79÷0.55=1.4
Sea water containing 3.2 per cent formaldehyde	8.37−7.60=0.77	0.77÷0.55=1.4
Sea water containing 3.2 per cent formaldehyde	8.37−7.65=0.72	0.72÷0.55=1.3
Sea water containing 3.2 per cent formaldehyde	8.37−7.70=0.67	0.67÷0.55=1.2
Sea water containing 3.2 per cent formaldehyde	8.37−7.80=0.57	0.57÷0.55=1.0
Sea water containing 3.2 per cent formaldehyde	8.37−7.85=0.52	0.52÷0.55=0.95

TABLE II A CONTROL

FIFTEEN PERIODS (21 MIN.) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1	8.37−7.80=0.57	0.57÷0.52=1.10
2	8.37−7.90=0.47	0.47÷0.52=0.90
3	8.37−7.90=0.47	0.47÷0.52=0.90
4	8.37−7.90=0.47	0.47÷0.52=0.90
5	8.37−7.92=0.45	0.45÷0.52=0.87
6	8.37−7.92=0.45	0.45÷0.52=0.87
7	8.37−7.92=0.45	0.45÷0.52=0.87
8	8.37−7.92=0.45	0.45÷0.52=0.87
9	8.37−7.90=0.47	0.47÷0.52=0.90
10	8.37−7.90=0.47	0.47÷0.52=0.90
11	8.37−7.90=0.47	0.47÷0.52=0.90
12	8.37−7.90=0.47	0.47÷0.52=0.90
13	8.37−7.90=0.47	0.47÷0.52=0.90
14	8.37−7.90=0.47	0.47÷0.52=0.90
15	8.37−7.90=0.47[1]	0.47÷0.52=0.90

TABLE II B

TWO PERIODS (22.25 MIN. EACH) IN SEA WATER FOLLOWED BY 5 EQUAL PERIODS IN SEA WATER CONTAINING 0.8 PER CENT FORMALDEHYDE (2 PER CENT BY VOLUME OF 40 PER CENT FORMALDEHYDE)

Solution	Change in PH	Relative rate of respiration
Sea water	7.90−7.55=0.35	
" "	7.90−7.55=0.35	
Sea water containing 0.8 per cent formaldehyde	7.90−6.81=1.09	1.09÷0.35=3.1
Sea water containing 0.8 per cent formaldehyde	7.90−6.96=0.94	0.94÷0.35=2.7
Sea water containing 0.8 per cent formaldehyde	7.90−7.03=0.87	0.87÷0.35=2.5
Sea water containing 0.8 per cent formaldehyde	7.90−6.95=0.95	0.95÷0.35=2.7
Sea water containing 0.8 per cent formaldehyde	7.90−6.95=0.95	0.95÷0.35=2.7

TABLE II B CONTROL

FIVE PERIODS (20 MIN.) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1	7.90−7.50=0.40	0.40÷0.40=1.00
2	7.90−7.50=0.40	0.40÷0.40=1.00
3	7.90−7.55=0.35	0.35÷0.40=0.87
4	7.90−7.55=0.35	0.35÷0.40=0.87
5	7.90−7.55=0.35	0.35÷0.40=0.87

TABLE II C*

TWO PERIODS (35.5 MIN. EACH) IN SEA WATER AND DURING 6 EQUAL PERIODS IN SEA WATER CONTAINING 0.3 PER CENT (BY VOLUME) OF CHLOROFORM

Solution	Change in PH	Relative rate of respiration
Sea water	8.03−7.43 =0.60	
" "	8.03−7.40 =0.63	
Sea water containing 0.3 per cent chloroform	8.03−6.30† =1.73	1.73÷0.615=2.8
Sea water containing 0.3 per cent chloroform	8.03−6.78 =1.25	1.25÷0.615=2.0
Sea water containing 0.3 per cent chloroform	8.03−7.15 =0.88	0.88÷0.615=1.4
Sea water containing 0.3 per cent chloroform	8.03−7.55 =0.48	0.48÷0.615=0.78
Sea water containing 0.3 per cent chloroform	8.03−7.65 =0.38	0.38÷0.615=0.62
Sea water containing 0.3 per cent chloroform	8.03−7.85 =0.18	0.18÷0.615=0.29

* In the control, with 8 periods (31.5 min. each) in sea water, the change in PH in each period was 8.03−7.80=0.23.

† Approximate (at this point the indicator is not very sensitive to changes in PH).

TABLE II D*

ONE PERIOD (44.5 MIN.) IN SEA WATER FOLLOWED BY EQUAL PERIOD IN SEA WATER CONTAINING 0.5 PER CENT (BY VOLUME) OF CHLOROFORM

Solution	Change in PH	Relative rate of respiration
Sea water	8.10−7.35=0.75	
Sea water containing 0.05 per cent chloroform	8.10−6.97=1.13	1.13÷0.75=1.5

* In the control, with 2 periods (39.25 min. each) in sea water, the change in PH in each period was 8.10−7.35=0.75.

(fig. 2*a* [B], fig. 2*b* [B]; table II B) of formaldehyde respectively. In both figs. 2*a* and 2*b* (A) and (B), there is a marked increase in the respiration during the first period. The curves for the weaker concentration tend to become approximately horizontal in the later periods. The curves for the stronger concentration of formaldehyde present a somewhat different case. Here the respiration reaches its maximum during the first period and maintains this rate during the second period. Following this, the respiratory rate steadily becomes smaller.

The curves for 0.3 per cent (fig. 2*a* [C] and fig. 2*b* [C]; table II C) and 0.5 per cent (fig. 2*a* [D] and fig. 2*b* [D]; table II D) chloroform respectively, each show that respiration is increased during the first period. The curves for the 0.3 per cent chloroform indicate that the rate steadily becomes smaller, until at the end of about 2.25 hours the respiratory rate falls below what it was normally (when in sea water).

The observation has often been made (11) that in human beings and in mammals during prolonged anesthesia there are typical products of incomplete oxidation such as fatty acids, lactic acid, and above all acetone (in not inconsiderable quantities) eliminated, as the case may be, into the urine or into the respired air. It seemed of interest in this connection to study the effect of acetone upon the respiration of *Laminaria*. It will be seen from the curves that when sea water contains 0.1 per cent (fig. 3 [A]; table III A) or 0.51 per cent (fig. 3 [B]; table III B) of acetone respectively, the respiration is practically unaffected. When, however, the sea water contains 17.4 per cent of acetone (fig. 3 [C] and [D]; table III C), a peculiar condition results. During the first period the

respiratory rate (curve D) is greatly increased and reaches its maximum during the second period. This is followed by a rapid decrease in the rate during the third and fourth periods, although the rate is still above the normal. After the fourth period a more

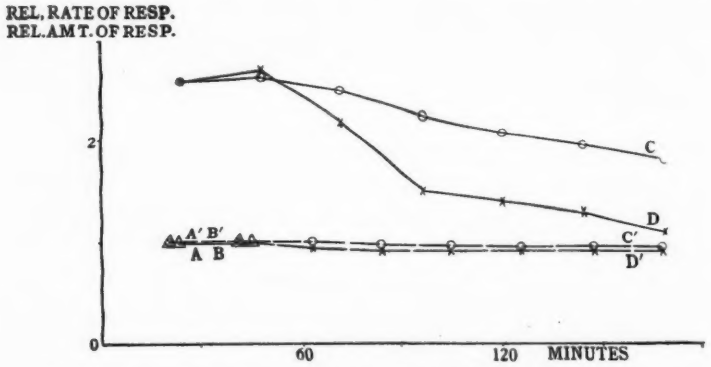


FIG. 3.—Curves (unbroken lines) showing effect of sea water containing *A*, 0.1 per cent acetone; *B*, 0.51 per cent acetone upon relative rate and relative amount of respiration of *Laminaria* (identical for these substances); *C*, effect of sea water containing 17.4 per cent acetone (unbroken line) upon relative amount of respiration; *D*, effect of sea water containing 17.4 per cent acetone (unbroken line) upon relative rate of respiration; controls in sea water (broken lines); see tables III A to III C; each control bears the same letter (with a prime) as the experimental curve.

gradual decline begins, so that even at the end of the experiment covering 2 hours and 48 min. the respiratory rate is still slightly above the normal.

TABLE III A AND B

TWO PERIODS (23 MIN. EACH) IN SEA WATER FOLLOWED BY 2 EQUAL PERIODS IN SEA WATER CONTAINING 0.1 PER CENT ACETONE, FOLLOWED BY 2 SIMILAR PERIODS IN SEA WATER CONTAINING 0.51 PER CENT ACETONE

Solution	Change in PH	Relative rate of respiration
Sea water	8.37−7.64=0.73	
" "	8.37−7.64=0.73	
Sea water containing 0.1 per cent acetone	8.37−7.65=0.72	0.72÷0.73=0.99
Sea water containing 0.1 per cent acetone	8.37−7.65=0.72	0.72÷0.73=0.99
Sea water containing 0.51 per cent acetone	8.37−7.65=0.72	0.72÷0.73=0.99
Sea water containing 0.51 per cent acetone	8.37−7.63=0.74	0.74÷0.73=1.01

TABLE III A AND B CONTROL

SIX PERIODS (20.25 MIN. EACH) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1	8.37−7.60=0.77	0.77÷0.77=1.00
2	8.37−7.60=0.77	0.77÷0.77=1.00
3	8.37−7.61=0.76	0.76÷0.77=0.99
4	8.37−7.60=0.77	0.77÷0.77=1.00
5	8.37−7.60=0.77	0.77÷0.77=1.00
6	8.37−7.60=0.77	0.77÷0.77=1.00

TABLE III C

TWO PERIODS (24 MIN. EACH) IN SEA WATER AND 7 EQUAL PERIODS IN SEA WATER CONTAINING 17.4 PER CENT (BY VOLUME) OF ACETONE

Solution	Change in PH	Relative rate of respiration
Sea water	8.37−7.76=0.61	
" "	8.37−7.78=0.59	
Sea water containing 17.4 per cent acetone	8.37−6.80=1.57	1.57÷0.60=2.6
Sea water containing 17.4 per cent acetone	8.37−6.75=1.62	1.62÷0.60=2.7
Sea water containing 17.4 per cent acetone	8.37−7.06=1.31	1.31÷0.60=2.2
Sea water containing 17.4 per cent acetone	8.37−7.50=0.87	0.87÷0.60=1.5
Sea water containing 17.4 per cent acetone	8.37−7.54=0.83	0.83÷0.60=1.4
Sea water containing 17.4 per cent acetone	8.37−7.62=0.75	0.75÷0.60=1.3
Sea water containing 17.4 per cent acetone	8.37−7.74=0.63	0.63÷0.60=1.1

TABLE III C CONTROL

NINE PERIODS (21 MIN. EACH) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1	8.37−7.67=0.70	0.70÷0.70=1.00
2	8.37−7.67=0.70	0.70÷0.70=1.00
3	8.37−7.70=0.67	0.67÷0.70=0.95
4	8.37−7.73=0.64	0.64÷0.70=0.91
5	8.37−7.73=0.64	0.64÷0.70=0.91
6	8.37−7.73=0.64	0.64÷0.70=0.91
7	8.37−7.72=0.65	0.65÷0.70=0.93
8	8.37−7.73=0.64	0.64÷0.70=0.91
9	8.37−7.72=0.65	0.65÷0.70=0.93

In view of the fact that alcohol is considered to be formed during respiration, it was deemed important to study the effect of varying concentrations of alcohol upon the rate of respiration. When sea water contains 1 per cent of Squibb's absolute alcohol (figs. 4*a* [F] and 4*b* [F]; table IV F), the respiratory rate remains normal for 3 periods, after which there is a gradual decline to below

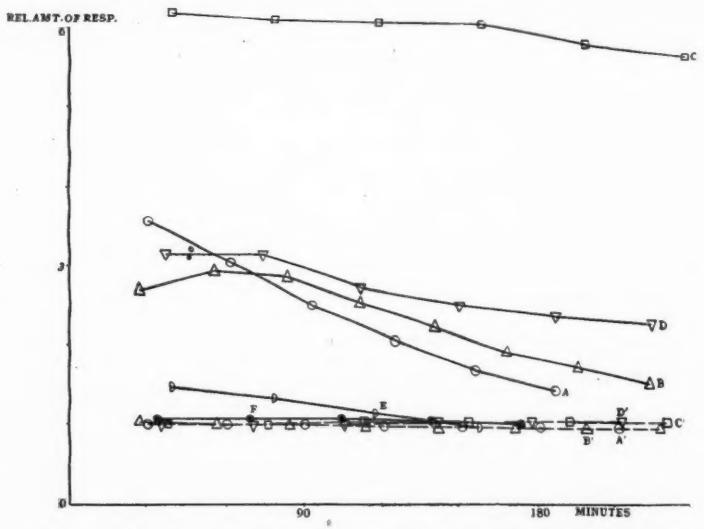


FIG. 4*a*.—Curves (unbroken lines) showing effect upon relative amount of respiration of *Laminaria* of sea water containing *A*, 24.2 per cent ethyl alcohol; *B*, 16.1 per cent ethyl alcohol; *C*, 10 per cent ethyl alcohol; *D*, 5 per cent ethyl alcohol; *E*, 2 per cent ethyl alcohol; *F*, 1 per cent ethyl alcohol; controls in sea water (broken lines); each control bears same letter (with prime) as the experimental curve, except that *D'* serves as control for curves *D*, *E*, and *F*; see tables IV A to IV F.

the normal rate, whereas the relative amount of respiration remains nearly constant. The curves for sea water containing 2 per cent alcohol (figs. 4*a* [E] and 4*b* [E]; table IV E) show a slight increase in the relative rate during the first period, followed by a smaller increase for the second period, after which there is a decline below the normal. As would be expected, 5 per cent alcohol (figs. 4*a* [D] and 4*b* [D]; table IV D) gives a much greater increase in the rela-

tive respiratory rate and amount than does 2 per cent alcohol (curve E). Unlike the 2 per cent alcohol, the maximum increase is maintained during the second period. The rate becomes less during the third period, after which it becomes quite constant. The curves for 10 per cent alcohol (figs. 4*a* [C] and 4*b* [C]; table IV C) reach their maximum during the first period. Approximately the

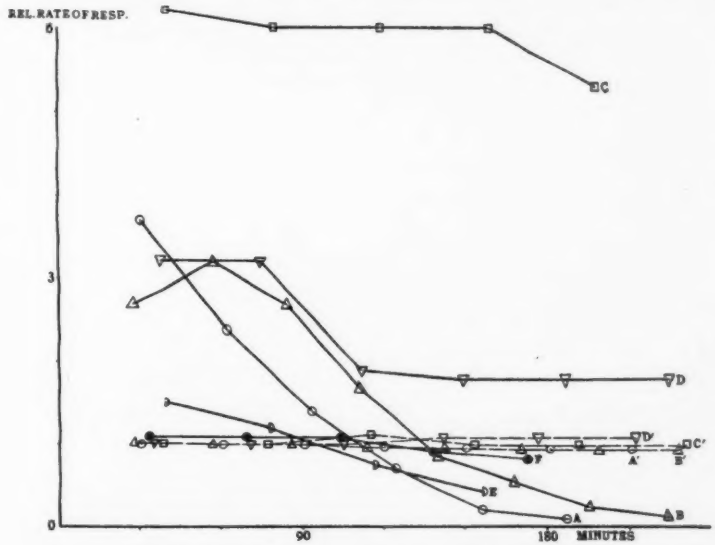


FIG. 4*b*.—Curves (unbroken lines) showing effect upon relative rate of respiration of *Laminaria* of sea water containing *A*, 24.2 per cent ethyl alcohol; *B*, 16.1 per cent ethyl alcohol; *C*, 10 per cent ethyl alcohol; *D*, 5 per cent ethyl alcohol; *E*, 2 per cent ethyl alcohol; *F*, 1 per cent ethyl alcohol; controls in sea water (broken lines); each control bears same letter (with prime) as the experimental curve, except that *D'* serves as control for curves *D*, *E*, and *F*; see tables IV A to IV F.

same rate of respiration is maintained for 3 periods, after which the increased rate rapidly becomes smaller. The relative amount of respiration remains approximately constant for 4 periods and then falls off very gradually. With 16.1 per cent alcohol (figs. 4*a* [B] and 4*b* [B]; table IV B) the maximum rate is not reached until the second period, after which the decline is more rapid than that for any of the lower concentrations. It will be seen that at the end of

224 min. the relative amount is about 1.5, while the relative rate falls below the normal after about 130 min. The maximum increased rate, when 24.2 per cent alcohol is used (figs. 4*a* [A] and 4*b* [A]; table IV A), is reached during the first period and then becomes smaller very much more rapidly than at any of the lower

TABLE IV A

TWO PERIODS (31.25 MIN. EACH) IN SEA WATER AND 6 EQUAL PERIODS IN SEA WATER CONTAINING 24.2 PER CENT (BY VOLUME) OF ETHYL ALCOHOL

Solution	Change in PH	Relative rate of respiration
Sea water	7.90−7.43 =0.47	.....
" "	7.90−7.43 =0.47	.....
Sea water containing 24.2 per cent ethyl alcohol	7.90−6.16*=1.74	1.74÷0.47=3.7
Sea water containing 24.2 per cent ethyl alcohol	7.90−6.75 =1.15	1.15÷0.47=2.4
Sea water containing 24.2 per cent ethyl alcohol	7.90−7.25 =0.65	0.65÷0.47=1.4
Sea water containing 24.2 per cent ethyl alcohol	7.90−7.55 =0.35	0.35÷0.47=0.7
Sea water containing 24.2 per cent ethyl alcohol	7.90−7.80 =0.10	0.10÷0.47=0.2
Sea water containing 24.2 per cent ethyl alcohol	7.90−7.84 =0.06	0.06÷0.47=0.1

* Approximate (at this point the indicator is not very sensitive to changes in PH).

TABLE IV A CONTROL

EIGHT PERIODS (30.25 MIN. EACH) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1	7.90−7.53=0.37	0.37÷0.37=1.0
2	7.90−7.53=0.37	0.37÷0.37=1.0
3	7.90−7.53=0.37	0.37÷0.37=1.0
4	7.90−7.54=0.36	0.36÷0.37=0.97
5	7.90−7.54=0.36	0.36÷0.37=0.97
6	7.90−7.55=0.35	0.35÷0.37=0.95
7	7.90−7.55=0.35	0.35÷0.37=0.95
8	7.90−7.58=0.32	0.32÷0.37=0.87

concentrations, falling below the normal rate at about 110 min. The curve for relative amount of respiration at the end of 3 hours is about 1.4, even though respiration has nearly ceased.

These curves, showing the effect of varying concentrations of alcohol upon the rate of respiration, indicate that for concentrations above 1 per cent there is a marked increase in the respiratory

activity. Furthermore, there is a maximum increase which is usually reached in the first, but sometimes not until the second period. The relative amount and relative rate of respiration increase with increasing concentrations of ethyl alcohol up to 10

TABLE IV B

TWO PERIODS (28 MIN. EACH) IN SEA WATER FOLLOWED BY 8 EQUAL PERIODS IN SEA WATER CONTAINING 16.1 PER CENT ETHYL ALCOHOL

Solution	Change in PH	Relative rate of respiration
Sea water	7.95−7.50=0.45	
" "	7.95−7.52=0.43	
Sea water containing 16.1 per cent ethyl alcohol	7.95−6.76=1.19	1.19÷0.44=2.7
Sea water containing 16.1 per cent ethyl alcohol	7.95−6.55=1.40	1.4 ÷0.44=3.2
Sea water containing 16.1 per cent ethyl alcohol	7.95−6.75=1.20	1.2 ÷0.44=2.7
Sea water containing 16.1 per cent ethyl alcohol	7.95−7.20=0.75	0.75÷0.44=1.7
Sea water containing 16.1 per cent ethyl alcohol	7.95−7.57=0.38	0.38÷0.44=0.86
Sea water containing 16.1 per cent ethyl alcohol	7.95−7.70=0.25	0.25÷0.44=0.57
Sea water containing 16.1 per cent ethyl alcohol	7.95−7.85=0.10	0.10÷0.44=0.23
Sea water containing 16.1 per cent ethyl alcohol	7.95−7.90=0.05	0.05÷0.44=0.11

TABLE IV B CONTROL

NINE PERIODS (28.5 MIN. EACH) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1	7.95−7.55=0.40	0.40÷0.395=1.01
2	7.95−7.50=0.39	0.39÷0.395=0.99
3	7.95−7.56=0.39	0.39÷0.395=0.99
4	7.95−7.57=0.38	0.38÷0.395=0.96
5	7.95−7.58=0.37	0.37÷0.395=0.94
6	7.95−7.58=0.37	0.37÷0.395=0.94
7	7.95−7.58=0.37	0.37÷0.395=0.94
8	7.95−7.58=0.37	0.37÷0.395=0.94
9	7.95−7.58=0.37	0.37÷0.395=0.94

per cent. At concentrations from 2 to 10 per cent the relative rate of respiration remained far above the normal during the entire experiment. When larger concentrations such as 16.1 or 24 per cent are used, however, the decline, once the maximum rate has been reached, becomes more rapid with increasing concentrations

of alcohol. The curves of the relative rate or respiration for such higher concentrations of alcohol fall quite rapidly below the normal, whereas the curves for the relative amount of respiration for the same experiment may remain far above unity.

TABLE IV C

TWO PERIODS (39.75 MIN. EACH) IN SEA WATER FOLLOWED BY 6 EQUAL PERIODS IN SEA WATER CONTAINING 10 PER CENT ETHYL ALCOHOL

Solution	Change in PH	Relative rate of respiration
Sea water	7.70−7.55=0.15	
" "	7.70−7.55=0.15	
Sea water containing 10 per cent ethyl alcohol	7.70−6.77=0.93	0.93÷0.15=6.2
Sea water containing 10 per cent ethyl alcohol	7.70−6.80=0.90	0.90÷0.15=6.0
Sea water containing 10 per cent ethyl alcohol	7.70−6.80=0.90	0.90÷0.15=6.0
Sea water containing 10 per cent ethyl alcohol	7.70−6.80=0.90	0.90÷0.15=6.0
Sea water containing 10 per cent ethyl alcohol	7.70−6.90=0.80	0.80÷0.15=5.3
Sea water containing 10 per cent ethyl alcohol	7.70−7.05=0.65	0.65÷0.15=4.3

TABLE IV C CONTROL

EIGHT PERIODS (38.5 MIN. EACH) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1	7.70−7.55=0.15	0.15÷0.15=1.00
2	7.70−7.55=0.15	0.15÷0.15=1.00
3	7.70−7.53=0.17	0.17÷0.15=1.13
4	7.70−7.55=0.15	0.15÷0.15=1.00
5	7.70−7.55=0.15	0.15÷0.15=1.00
6	7.70−7.55=0.15	0.15÷0.15=1.00
7	7.70−7.55=0.15	0.15÷0.15=1.00
8	7.70−7.55=0.15	0.15÷0.15=1.00

It is important to know whether or not the decrease in PH value is due to the excretion of CO_2 or to other acids, such as organic acids which are products of incomplete oxidation. To determine this pure hydrogen was bubbled through the solution (which has been made more acid by *Laminaria*) until the excess of CO_2 was expelled. The solution was then allowed to come into equilibrium with the CO_2 of the air. It was found that in all the experiments

(with certain exceptions to be mentioned) the color of the indicator was reversible by this means. This showed conclusively that the increased acidity was actually due to CO_2. In the case of sea water containing 0.3 per cent chloroform, the color of the indicator

TABLE IV D

TWO PERIODS (37.5 MIN. EACH) IN SEA WATER FOLLOWED BY 6 EQUAL PERIODS IN SEA WATER CONTAINING 5 PER CENT ETHYL ALCOHOL

Solution	Change in PH	Relative rate of respiration
Sea water	7.65−7.43=0.22	
" "	7.65−7.43=0.22	
Sea water containing 5 per cent ethyl alcohol	7.65−6.95=0.70	0.70÷0.22=3.2
Sea water containing 5 per cent ethyl alcohol	7.65−6.95=0.70	0.70÷0.22=3.2
Sea water containing 5 per cent ethyl alcohol	7.65−7.22=0.43	0.43÷0.22=1.9
Sea water containing 5 per cent ethyl alcohol	7.65−7.25=0.40	0.40÷0.22=1.8
Sea water containing 5 per cent ethyl alcohol	7.65−7.25=0.40	0.40÷0.22=1.8
Sea water containing 5 per cent ethyl alcohol	7.65−7.25=0.40	0.40=0.22=1.8

TABLE IV E

TWO PERIODS (39.25 MIN. EACH) IN SEA WATER FOLLOWED BY 4 EQUAL PERIODS IN SEA WATER CONTAINING 2 PER CENT ETHYL ALCOHOL

Solution	Change in PH	Relative rate of respiration
Sea water	7.65−7.35=0.30	
" "	7.65−7.35=0.30	
Sea water containing 2 per cent ethyl alcohol	7.65−7.20=0.45	0.45÷0.30=1.5
Sea water containing 2 per cent ethyl alcohol	7.65−7.28=0.37	0.37÷0.30=1.2
Sea water containing 2 per cent ethyl alcohol	7.65−7.43=0.22	0.22÷0.30=0.73
Sea water containing 2 per cent ethyl alcohol	7.65−7.52=0.13	0.13÷0.30=0.43

was not reversible at the end of the first period, although after any of the succeeding periods the color was fully reversible. The color of the indicator in sea water containing 17.4 per cent acetone (made up to the conductivity of sea water) was not reversible at the end of the first period of exposure. At the end of the second period of

exposure the color was almost entirely reversible, and after the third period (and succeeding periods) the color was completely reversible. When sea water containing 24.2 per cent ethyl alcohol (made up to conductivity of sea water) was used, the color of the indicator after the first period of exposure was not reversible. The

TABLE IV F

TWO PERIODS (34.75 MIN. EACH) IN SEA WATER FOLLOWED BY 5 EQUAL PERIODS IN SEA WATER CONTAINING 1 PER CENT ETHYL ALCOHOL

Solution	Change in PH	Relative rate of respiration
Sea water	7.65−7.40=0.25	
" "	7.65−7.35=0.30	
Sea water containing 1 per cent ethyl alcohol	7.65−7.35=0.30	0.30÷0.275=1.1
Sea water containing 1 per cent ethyl alcohol	7.65−7.35=0.30	0.30÷0.275=1.1
Sea water containing 1 per cent ethyl alcohol	7.65−7.35=0.30	0.30÷0.275=1.1
Sea water containing 1 per cent ethyl alcohol	7.65−7.40=0.25	0.25÷0.275=0.91
Sea water containing 1 per cent ethyl alcohol	7.65−7.43=0.22	0.22÷0.275=0.80

TABLE IV D, E, F CONTROL

SIX PERIODS (35.5 MIN. EACH) IN SEA WATER

Period	Change in PH	Relative rate of respiration
1	7.65−7.45=0.20	0.20÷0.20=1.00
2	7.65−7.45=0.20	0.20÷0.20=1.00
3	7.65−7.45=0.20	0.20÷0.20=1.00
4	7.65−7.43=0.22	0.22÷0.20=1.10
5	7.65−7.43=0.22	0.22÷0.20=1.10
6	7.65−7.43=0.22	0.22÷0.20=1.10

color of the indicator at the end of the second period of exposure was almost completely reversible, but after each subsequent period the color was fully reversible.

The reason for these instances of irreversibility can easily be explained. Phenolsulphonephthalein in the acid end of its range is yellow. *Laminaria* has a yellowish brown pigment which may come out when the cells are injured by very strong concentrations of the anesthetic, while the green pigment does not come out. When the concentration of the anesthetic is so great as to rapidly injure the cells (as is the case with all of the exceptions just noted),

the yellowish pigment comes out so rapidly as to interfere with the indicator. As soon as the extraction of the pigment ceases (usually ending during the first 2 periods), the color of the indicator is not interfered with. When the concentrations of anesthetics are lower than in those instances just mentioned, the pigment comes out (if at all) so slowly as not to affect the indicator. This can be proven by matching the solution obtained after any period (without the addition of indicator) with the color of sea water (containing no indicator) in a tube of equal diameter. In such cases no pigment is detectable.

Further evidence that it is CO_2 that is being measured in the experiments rather than other acids is the fact that by the use of the gas chain it was found that *Laminaria*, after being 2 weeks in a small quantity of unchanged sea water, had given off no acid other than CO_2.

It might be supposed that the addition of so much alcohol as 24.2 per cent would dilute the buffer substances of the sea water so that a given amount of CO_2 added to the mixture would produce more change in PH value than would be the case in sea water. This was largely avoided by concentrating the sea water before adding the alcohol, so that the amount of buffer substance remained the same in the mixture as in the sea water alone. Tests made by adding measured amounts of CO_2 to sea water and to sea water plus 24.2 per cent alcohol (made up to the electrical conductivity of sea water) showed that there was not sufficient difference in this respect to be of importance in this investigation.

Such experiments enable us to follow the respiration of the same piece of tissue during shorter or longer periods of exposure to various concentrations of anesthetics. They further show that in no instance was there an initial decrease in the rate of respiration.

It will be observed that when the concentration of anesthetic is strong enough to produce any measurable result, the first effect is an increase of respiration, which gradually declines and may eventually fall below the normal. This decline is interpreted by the writer as a toxic effect.

These results are not in accord with the statements of TASHIRO and ADAMS (**27**), according to whom anesthetics do not produce

an increase of respiration except when their concentration is so low that they have only a stimulating action. They state that when the concentration is increased to the point where anesthesia occurs, the rate of respiration falls below the normal.

It is evident that this is not the case with *Laminaria*, for in no instance was the respiration observed to fall below the normal except after prolonged exposure to high concentrations which produced death. Further investigation will be necessary to determine the cause of these discrepancies.

It is evident that these experiments directly contradict the idea, advocated by VERWORN (**29**) and his pupils, that anesthesia is a kind of asphyxia and that anesthetics act by reducing respiration.

Summary

When *Laminaria* is exposed to anesthetics (in sufficiently high concentration to produce any result) the initial effect is an increase of respiration. This may be followed by a decrease if the anesthetic is sufficiently toxic. No decrease of respiration is observed when the concentration is too low to be toxic.

These results directly contradict the idea advocated by VERWORN and his pupils that anesthetics act by decreasing respiration.

LABORATORY OF PLANT PHYSIOLOGY
HARVARD UNIVERSITY

LITERATURE CITED

1. APPLEMAN, C. O., Relation of oxidases and catalase to respiration in plants. Amer. Jour. Bot. **3**:223. 1916.
2. BATTELLI, F., and STERN, L., Einfluss der Anästhetika auf die Oxydone. Biochem. Zeitschr. **52**:226. 1913.
3. BONNIER, G., and MANGIN, L., Recherches sur l'action chlorophylliene séparée de la respiration. Ann. Sci. Nat. VII. **3**:5. 1886.
4. BÜRKER, K., Eine neue Theorie der Narkose. Münch. Mediz. Wochenschr. **57**:1443. 1910.
5. CZAPEK, F., Biochemie der Pflanzen. 1913 (195 ff.).
6. EWART, A. J., The action of chloroform on CO_2 assimilation. Ann. Botany **12**:415. 1898.
7. HAAS, A. R., A simple and rapid method of studying respiration by the detection of exceedingly minute quantities of carbon dioxide. Science N.S. **44**:105. 1916.

8. HAMBURGER, E., Narkose und Sauerstoffmangel. Pflüger's Arch. **143**: 186. 1912.
9. HARDER, R., Beiträge zur Kenntniss des Gaswechsels der Meeresalgen. Jahrb. Wiss. Bot. **56**:254. 1915.
10. HÖBER, R., Physik. Chem. der Zelle und der Gewebe, Chaps. 8, 9. 1914.
11. IRVING, A. A., The effect of chloroform upon respiration and assimilation. Ann. Botany **25**:1077. 1912.
12. KOSINSKI, I., Die Athmung bei Hungerständen und unter Einwirkung von mechanischen und chemischen Reizmitteln bei *Aspergillus niger*. Jahrb. Wiss. Bot. **37**:137. 1902.
13. LAURÉN, W., Über den Einfluss von Aetherdämpfen auf die Atmung von Keimlingen. Diss. Helsingfors 1891. Cited from Just's Bot. Jahresb. **20**:92. 1892.
14. LOEB, J., and WASTENEYS, H., Is narcosis due to asphyxiation? Jour. Biol. Chem. **14**:517. 1913.
15. MCCLENDON, J. F., The composition, especially the hydrogen ion concentration, of sea water in relation to marine organisms. Jour. Biol. Chem. **28**:135. 1916.
16. MANSFELD, G., Narkose und Sauerstoffmangel. Pflüger's Arch. **129**:69. 1909.
17. MATTHAEI, G. L. C., Experimental researches on vegetable assimilation and respiration. III. On the effect of temperature on carbon dioxide assimilation. Trans. Roy. Soc. London B. **197**:71. 1904.
18. MAYER, A., Landw. Versuchsstat. 1879. S. 335.
19. MEYER, H. H., Zur Theorie der Alkoholnarcose. Welche Eigenschaft der Anesthetica bedingt ihre narcotische Wirkung? Arch. Exper. Pathol. **42**:109. 1899.
20. NOTHMANN-ZUCKERANDL, H., Die Wirkung der Narkotica auf die Plasmaströmung. Biochem. Zeitschr. **45**:412. 1912.
21. OVERTON, E., Studien über die Narkose. 1901. S. 51.
22. PANTANELLI, E., Athmung der Meeresalgen. Ber. Deutsch. Bot. Gesells. **32**:488. 1914.
23. RICHARDS, H. M., The respiration of wounded plants. Ann. Botany **10**:551. 1896; The evolution of heat by wounded plants. Ann. Botany **11**:29. 1897.
24. SCHROEDER, H., Über den Einfluss des Cyankaliums auf die Atmung von *Aspergillus niger* nebst Bemerkungen über die Mechanik der Blausäure-Wirkung. Jahrb. Wiss. Bot. **44**:409. 1907.
25. SÖRENSEN, S.P.L., Compt. Rend. Lab. Carlsberg **8**:1. 1909.
26. TASHIRO, S., Carbon dioxide production from nerve fibers when resting and when stimulated; a contribution to the chemical basis of irritability. Amer. Jour. Physiol. **32**:107. 1913.
27. TASHIRO, S., and ADAMS, H. S., Studies in narcosis. I. Effect of ethyl urethane and chloral hydrate on the CO_2 production of the nerve fiber. Internat. Zeitschr. Phys.-Chem. Biol. **1**:450. 1914.

28. VERNON, H. M., The function of lipoids in tissue respiration and in the activity of oxidases. Jour. Physiol. **45**:197. 1912; Die Abhangigkeit der Oxydasewirkung von Lipoiden. Biochem. Zeitschr. **47**:374. 1912.

29. VERWORN, M., Irritability. 1913 (Chap. IX).

30. WARBURG, O., Über Beeinflussung der Sauerstoffatmung. Zeitschr. Physiol. Chemie. **70**:413. 1911; see also Münch. Mediz. Wochenschr. 1911, No. 6; Oxydationen in lebenden zellen nach Versuchen an rothen Blutkörperchen. Zeitschr. Physiol. Chemie **66**:305. 1910.

31. WINTERSTEIN, H., Beitrage zur Kenntniss der Narkose. I Mittheilung. Kritische Übersicht über die Beziehungen zwischen Narkose und Sauerstoffatmung. *Biochem. Zeitschr.* **51**:143. 1913.

BASIS OF SUCCULENCE IN PLANTS

D. T. MacDougal, H. M. Richards, and H. A. Spoehr

Succulents may be characterized as plants in which the parenchymatous elements show an exaggerated development with relation to the more rigid tissues, and, unlike pith or medullary tracts, the masses of thin-walled cells remain distended and turgid. The liquid contents of such cells may or may not contain much dissolved material. The disposition of the water-holding tracts varies from leaves to stems and roots, but in all cases the most important general effect is one of massiveness, and the surfaces of succulent plants may in such forms as the barrel cacti bear the smallest possible proportion to the mass, that of a globe.

The ecologist recognizes two general types of succulents, those of the arid regions, which are of a xerophytic character, exemplified by the cacti; and the halophytes or fleshy seashore plants, also at home in alkaline areas. The plants of the two types are quite unlike in their transpiratory relations. The desert succulents may lose water so slowly that an existence of several years may be maintained upon the water in the thin-walled tracts.[1] On the other hand, the halophytes or fleshy shore plants may flag and wilt as readily as any thin-leaved form, due to the rapid loss of water from the surfaces. The origination of these striking forms has been the subject of much speculation, but all attempts to connect succulency in a causal way with the presence of salts in the soil, or in the plant with the well known high acidity of many of these forms, or with any purposeful development of water storage capacity, have been inadequate.

Our concurrent observations and experiments may be briefly summarized as follows:

1. A *Castilleja* native to the region about the Coastal Laboratory, at Carmel, California, includes two habitat forms, genetically identical, one with thin leaves growing in the open forest formation,

[1] MacDougal, D. T., et al., End results of desiccation and respiration in succulent plants. Physiol. Researches 1: 289–325. 1915.

and another with fleshy leaves growing on the sandy foreshores under arid, but not saline, soil conditions. The succulent leaves owe their increased thickness to the enlargement of elongated cells vertical to the surface of the leaves.

2. The thin leaves show an acidity double that of the fleshy type, and have a relatively greater dry weight.

3. The fleshy leaves, fresh and in a dried condition, present swelling reactions similar to those of sections of the joints of platyopuntias, indicative of cells high in pentosans, or mucilages. The behavior of these organs is different in many important particulars from that of thin leaves, which swell more in acid than in alkaline solutions, the reverse taking place in succulent leaves, in parallelism to *Opuntia*.

4. Differences in the swelling reactions of dried leaves of both kinds are to be ascribed to the adsorption of the contained acids and salts of different amounts in the two cases on cell colloids, high in pentosans in one case and hence presenting characteristic coagulatory effects.

5. It has been established by researches not described in this paper that the reduction of the water content of the cell below a certain point results in the conversion of polysaccharides, which do not show a high imbibition capacity, to pentosans, which mixed with nitrogenous substances have an enormous hydration capacity.

6. Succulence, therefore, may originate as it is seen to occur in *Castilleja* as a direct result of aridity. Species of *Ericameria* and *Erigeron* with a distribution similar to *Castilleja* display thin and succulent leaves corresponding in the same manner to the environment.

7. High acidity may not be taken as a result of succulence. It is probably more nearly correct to assume that succulence may develop only in plants which have a carbohydrate metabolism characterized by large acid residues.

The bulk and durability of succulents have made them readily available for chemical studies, and these features are responsible in part for the fact that carbohydrate metabolism and respiration, photosynthesis, the formation and fate of acids, the oxygen-carbon-dioxide ratio, and other features have all received contributions

based upon researches carried out with the fleshy plants.[2] The amount of detailed and systematic information concerning these plants as a type is probably greater than that of any other ecological group, and it is evident that their metabolism presents some definite characteristic aspects. It is upon the basis of such knowledge that it becomes possible to formulate the generalizations set forth in this paper.

The only comparisons between succulents and non-succulents that have been possible have lacked directness because the reactions of different species could not be rated against each other with accuracy. The final and necessary conditions for a critical discussion of the matter, that of succulent and non-succulent individuals of the same species, finally came to the attention of the authors in the case of *Castilleja latifolia* at Carmel, California, in the summer of 1918. One form of this plant which grows on the edge of the bluff overlooking the beach, or within 25 ft. of it, has succulent leaves of considerable thickness which are usually pale green. The other, which grows farther back on the foreshore, has a thinner, mesophytic type of leaf which is darker green and more hirsute than the succulent type. This is probably the typical form of the manuals and is similar to the one which grows farther back in the pine woods. A notable exception as to the relative region of growth of these 2 forms was found in a luxuriant growth of the thin-leaved type at the base of the beach bluff on the edge of the sand. Examination showed that this was unquestionably due simply to the ample water supply from the seepage at the base of the cliff. It becomes evident, therefore, that the difference in the succulent and mesophytic habit is not a case of even partial halophytism, for if salt were present anywhere it would be at the cliff base. The contrasting habit is one rather of xerophytism versus a mesophytic growth.

The members of the genus are reported to be parasitic, and individuals with thin and others with succulent leaves were found

[2] SPOEHR, H. A., Photochemische Vorgänge bei der Diurnalen Entsäurung der Succulenten. Biochem. Zeitsch. **57**:95–111. 1913.

RICHARDS, H. M., Acidity and gas interchange in cacti. Publ. no. 209. Carnegie Inst. Wash. 1915.

HEMPEL, JENNY, Buffer process in the metabolism of succulent plants. Compt. Rend. Carlsberg. **13**:no. 1. 1917.

with minute roots attached to the older tapering roots of *Artemisia pycnocephala*, in a manner indicating that the dependent nutritive relation is not an important one. That the appearance of succulence in this plant has no connection with its parasitism is supported by the fact that a similar state was found in *Erigeron glaucus* and *Ericameria ericoides* which are found in the locality near *Castilleja*.

Measurements of the thickness of the leaves of the two types show that the thin leaf averages about 0.5 mm., while the succulent one ranges from 1 to 1.5 mm. Examination of sections shows that the structure of the leaves is mainly differentiated by the size of the cells. While nearly dorsiventral in position when young, structurally the leaves appear almost bifacial, with 2 or 3 rows of vertically elongated cells on each face. In the thin-leaved type these cells are about $35 \times 25\ \mu$, while similar cells of the succulent leaf are $110 \times 30\ \mu$. Thus the increased thickness of the leaf is due chiefly to the enlargement of the cells vertical to the flat surface of the leaf.

There is naturally a disparity in the relation of fresh to dry weight in the 2 forms. Averages of a number of determinations show the following figures: mesophytic type, 1 gm. fresh young leaves, 0.193 dry weight; succulent type, 1 gm. fresh young leaves, 0.113 dry weight. In general the succulent type yields only three-fifths dry substance per unit fresh substance compared with the other form.

The acidity relations are also different. The succulent leaves are much less acid than the thinner ones. In table I averages of 10 or more determinations indicate the difference in acid extracted, and also the amount of water absorbed after 24 hours' immersion. As might be expected, the acidity relation of the 2 forms approximates more closely when reckoned according to dry weight than on the basis of fresh weight. Even then, however, the young active leaves show a considerably greater acidity in the thin type, a notable departure from our preconceived conceptions of acidity in relation to succulence. It may be said, however, that direct comparisons of acidity of a plant of the same species in a mesophytic and succulent condition do not seem to have been made.

In connection with the swelling measurements described later, it became important to ascertain how much acid would leach out during immersion in water. Table II indicates the averages of a considerable number of series.

It is noticeable that the total amount of acid is somewhat greater than the figures previously given, due very possibly to the formation of acid during immersion in water, which might be caused

TABLE I

Material	Total acidity		Water absorbed, cc. per gm. fresh weight
	Per gm. fresh	Per gm. dry	
Thin young leaves	1.50 cc. N/20 KOH	7.78 cc. N/20 KOH	1.14
Succulent young leaves	0.62 N/20	5.37 N/20	0.57
Thin old leaves	1.00 N/20	5.20 N/20	0.85
Succulent old leaves	0.65 N/20	5.74 N/20	0.35

TABLE II

Leaves immersed in water for 24 hours at 17° C.

Material	Acid diffused out per gm. fresh weight	Acid retained in tissue per gm. fresh weight
Thin young leaves	1.30 cc. N/20 KOH	0.39 cc. N/20 KOH
Succulent young leaves	0.55 N/20	0.28 N/20
Thin old leaves	0.62 N/20	0.66 N/20
Succulent old leaves	0.30 N/20	0.41 N/20

by the exclusion of oxygen. It is also to be observed that the residual acid in the young leaves is closely the same in both the succulent and thin-leaved type. It appears that in the old leaves proportionately less of the acid leaches out and more is retained in the tissues. Other series of experiments were undertaken to determine the rate at which the acid diffuses out, the results of which are given in table III.

During the first hours of immersion the amount of acid which passes out is small and nearly equal in each case. As prolonged immersion in water kills the leaves, it seems probable that very little acid escapes as long as the cells are alive.[3] Table IV indicates

[3] Lauk, E., Die Bedeutung der Elektrolyten fuer Quellungsprocess. Biochem. Zeitsch. **37**: 15–58. 1916.

the length of immersion which the 2 forms can withstand and recover to a seemingly normal condition.

After 12 hours' immersion in water all the thin-leaved shoots were killed; some of the succulent leaved shoots survived partly.

TABLE III

RATE OF DIFFUSION OF ACID IN TERMS OF CC. N/20 KOH PER GM. FRESH WEIGHT AT 17° C.

Material	3 hours	6 hours	9 hours	12 hours	15 hours	24 hours	Residual acid	Total
Thin young leaves.....	0.10	0.20	0.27	0.30	0.15	0.05	0.30	1.37
Succulent young leaves.....	0.10	0.12	0.18	0.15	0.05	Trace	0.23	0.80

TABLE IV

Material	1.5 hours	3 hours	6 hours	9 hours
Thin young leaves.....	All recovered	All recovered	Some killed	More than half killed
Succulent young leaves .	All recovered	All recovered	Some killed	About half killed

TABLE V

SWELLING OF LEAVES OF *Castilleja* AT 16° C.

Material	Water	Citric acid 0.01 N	Potassium hydrate 0.01 M	Potassium nitrate 0.01 M
	Percentage	Percentage	Percentage	Percentage
Thin.....	149	120	60	76
Succulent.....	143	95	125	90

The reactions of the succulent leaves are seen to present the general aspects of sections of joints of *Opuntia discata* grown at Carmel and tested at 16° C. at Carmel, July 1918. Swellings of dried slices are as follows:

Material	Water	Citric acid 0.01 N	Potassium hydrate 0.01 M	Potassium nitrate 0.01 M
	Percentage	Percentage	Percentage	Percentage
Fresh sections...	9	10	11.7	10.4
Dried slices.....	509	218	413	417

A set of sections were now prepared, and, after being swelled with reactions parallel to the preceding, were dried, and the expansion when immersed was calculated on the original thickness, as follows:

Material	Water	Citric acid 0.01 N	Potassium hydrate 0.01 M	Potassium nitrate 0.01 M
	Percentage	Percentage	Percentage	Percentage
Fresh sections ..	11.4	6.4	6.6	9.2
Dried sections ..	17.5	26.4	24.5	22.4

Determinations were also made of the escape of acid from dried leaves, which, as shown in table VI, is much more rapid than with the living material. It will be seen that the total of the acid extracted from the dried leaves is much less than that obtained from fresh leaves, which, as might be expected, indicates that some of the

TABLE VI

ESCAPE OF ACID FROM DRIED LEAVES IN TERMS OF CC. N/20 KOH PER GM. FRESH WEIGHT AT 17° C.

Material	2 hours	6 hours	12 hours	15 hours	24 hours	Residue	Total
Succulent young leaves..	0.30	0.10	0.5	Trace	Trace	0.12	0.57
Thin young leaves.....	0.54	0.20	0.8	Trace	Trace	0.40	1.22

acid salts are absorbed and held in the irreversible aggregation phenomena connected with the processes of drying. It may be mentioned that two series of both kinds of leaves, which by chance were dried much more slowly than the others, showed a difference in the rate at which they yielded up their acid; in both cases the amount which escaped in 2 hours was much less than in the case of the rapidly dried leaves.

When trios of leaves of the 2 types were placed under the auxograph to determine their unsatisfied hydration capacity, swellings as follows (table VII) were displayed at 16° C.

As will be seen by comparisons with data obtained from *Castilleja*, the dried mass behaves like the succulent leaves by showing but little expansion after immersion and drying.

Similar tests were applied to sections and to dried median slices of an unknown *Opuntia* which appeared to be less mucilaginous than *O. discata*. Dried slices came down to a thickness of

about 0.2 mm. and these gave swellings at 16° C. which are to be compared with the swellings of fresh material.

The second swelling produced an expansion scarcely more than half that of the first in all solutions, and being still further decreased in alkali, furnishing striking parallels with the action of succulent leaves of *Castilleja*.

TABLE VII

Process	Water	Citric acid 0.01 N	Potassium hydrate 0.01 M	Potassium nitrate 0.01 M
	Percentage	Percentage	Percentage	Percentage
After first drying	361	306	250	325
After second drying on basis of reduced thickness	42	56	100	75
Fresh sections through joints	9.7	7	.....	9.3

TABLE VIII

HYDRATION REACTIONS OF SUCCULENT AND THIN LEAVES OF *Castilleja*; JULY 28–31; AT 16° C.

Material	Water	Citric acid 0.01 N	Potassium hydrate 0.01 M	Potassium nitrate 0.01 M
	Percentage	Percentage	Percentage	Percentage
Succulent				
Fresh	143	95	125	90
Swelled and dried	20	16	20	60
Fresh dried	76	60	55	65
Thin leaves				
Fresh	140	120	60	76
Swelled and dried	132	12	67	153
Fresh dried	100	20	118	130

Two additional treatments of the leaves were given to test the effects of hydration on the swelling capacity of the contained colloids. In one case the trios of sections which had swelled were dried on filter paper for a day at 20° C., with only enough pressure to prevent warping or curling, then again hydrated in water or the identical solutions of the first swelling. The second case included a swelling of leaves which had been simply dried for a day at 20° C., in which process they came down to about half the original thickness. The measurements at 17–18° C., calculated on dried thickness, which was usually about one-half that of living material, are given in table VIII.

The comparisons which may be made upon the basis of such data are almost endless, and a citation of even the salient features of interest cannot be made briefly. The proportionate hydration of the succulent and thin leaves are reversed in acid and alkali. The succulent leaf, which proves to be one-half as acid as the thin leaf, swells most in the alkaline solution; while the thin leaves, with an acidity double that of the thick succulent ones, have an equivalent maximum in hundredth normal citric acid, and take up only half as much water in the alkaline solution, the disproportion between the two expansions being greater than that of the acid alkali ratio in the succulent.

The thin leaves are characterized by a uniformly high hydration capacity in water in the 3 cases, although reaching a maximum in the salt, a high swelling capacity in acid when fresh, which undergoes a great reduction after drying, while the swelling capacity increases in alkali in parallel treatments. The maximum swelling of the succulent leaves is in water, with great variation in the 3 conditions in which leaves were tested, and with but little variation in the reactions in the salt. The thin leaves, on the other hand, show the maximum and greatest diversity in the salts and more uniformity in water.

The variations in swelling in the acid solution presented such unusual features that an additional series was planned in which thin and succulent leaves in fresh condition were swelled, then such leaves fully hydrated in water and in various solutions were dried and swelled a second time for comparison with the reactions of leaves dried directly from the living condition. Table IX shows the swellings in 0.01 normal citric acid at 15° C.

The chief departure from the original series is in the matter of the swelling of the fresh succulent leaves, which in this case appear to have been in such a highly hydrated condition as only to be capable of slight expansion. This assumption is in accordance with the fact that after being immersed and then dried they assumed approximately the original thickness on a second swelling. The thin leaves of this series were consistent in their reactions with those previously examined, showing a relatively small expansion from a dried condition. The conditions making possible the greater variations are evidently those recognizable in the succulent type,

not the least important feature being the greatly enlarged parenchymatous cells. It is to be seen that immersion and drying, and also simple drying, reduce the swelling capacity of thin leaves in acid, but no such decrease occurs in the succulent leaf.

The principal changes which take place in swelling consist in the extraction of acids and acid salts, as indicated on the previous pages, and of the hexoses as yet undetermined. Any mucilages or pentosans present would of course diffuse at a rate so slow as to be of no consequence in the present experiments.

TABLE IX

Material	Thin		Succulent	
	Thickness	Swelling	Thickness	Swelling
	mm.	Percentage	mm.	Percentage
Fresh leaves	0.4	125	1.4	21
	0.41	184	1.4	25
Above leaves dried and rehydrated*	0.23	42	0.5–0.6	95
	0.25	20	0.63	91
Fresh dried leaves*	(0.38)	.....	(1.2)	.....
	0.2	25	0.5	120
	(0.38)	.....	(1.1)	.....
	0.2	62	0.38	92

* Expansion in terms of dried thickness.

The swelling of fresh leaves of both types in water reaches the limit in less than 2 hours, the rate of extraction of acid in the 2 types of leaves being equivalent, and the proportionate expansions not widely different. When such leaves are dried the thin leaves attain the limit in water inside of 2 hours, while the succulent leaves continue to expand for 6 hours with an escape of acid about half that from the thin leaf during the same time.

If attention be turned to the reactions in acids, it is seen that thin leaves swell more than succulents in such solutions when fresh, and that the swelling extends over a greater length of time, while the total swelling in a dried condition is accomplished in a few minutes. The succulent leaves, on the other hand, require a period of as much as 6 hours to reach full hydration from a dried condition.

While the effect of the residual acidity is discernible in some of these relations, it is evident that this factor is not the dominating

one. Two other features remain to be considered, that of the composition of the plasmatic colloids, and of the salts dissolved in the water of hydration. The colloids of living leaves are highly hydrated, and the salts, acids, etc., are also in a highly dilute condition, in which case their effect would be at a minimum. Death and desiccation would be accompanied by a concentration of these compounds, until finally they would be adsorbed by the cell walls and plasmatic colloids in their most concentrated condition with resulting coagulations, some of which in all probability are irreversible. The thin leaves have a higher acid content, and, to anticipate, a smaller proportion of pentosans which would accentuate this effect, hence the relatively low coefficient of swelling from a dried state. A long series of experiments with sections of dried colloids and of living and dried plants of known composition make it appear that the water relations of active tissues show the behavior of a biocolloid consisting largely of pentosans, of which agar or plant mucilages would be an example, a small proportion of protein or protein derivatives, and some salts and free acids.[4] It is to these features, therefore, that one would naturally turn for the factors which might increase the water-holding capacity of the cell or organ, and in so doing the pentosans would claim attention first. These substances probably are always present in some proportion in cells, and their occurrence is therefore not significant. Any action or condition which brings about a notable increase in their proportion in the cell would have most important consequences however. Such increase does result from a depletion of the water of a cell, for the polysaccharides under such conditions are reduced to the pentosans, and the reduction of the water content of a cell results in the conversion of the polysaccharides, which do not show marked imbibition, to pentosans, which take the form of an elastic gel with an enormous capacity for expansion, particularly when mixed with nitrogenous material, and upon this rests the hypertrophies or hyperplasias of thin-walled tracts in the development of

[4] MACDOUGAL, D. T., Imbibitional swelling of plants and colloidal mixtures. Science **44**:502. 1916.

MACDOUGAL D. T., and SPOEHR, H. A., The effect of acids and salts on biocolloids. Science **45**:269–272. 1917.

———, Growth and imbibition. Proc. Amer. Phil. Soc. **56**:289–352. 1917.

———, The behavior of certain gels useful in the interpretation of the action of plants. Science **45**:484–488. 1917.

succulence in an organ. Briefly restated, whenever the water content of a cell becomes low, some of the hexose-polysaccharides, which have a low imbibition capacity, are converted into pentosans, which have a high hydration capacity, the action having the force of a regulatory adjustment, and as the change is irreversible, the pentosans are accompanied by a permanent succulence, with all of the implied alterations in metabolism,[5] including a very striking change in the type of respiration, or of transformations in the carbohydrates.[6]

It is notable that, while this change in the sugars takes place in the cell, the type of transformations of energy changes completely, but the approximate rate of respiration is not materially affected. The nature and amount of the end products, however, may differ materially from those of a respiration in thin leaves, notably in the acid residues. It is in the mesh of reactions indicated that the origin and the nature of succulence will be found, and whatever causal value is attributed to the action of soil salts or of arid conditions will rest upon their part in the conversion of the polysaccharides to pentosans.

Acidity in succulents has been attributed by many writers, including the authors of this article, to the imperfect oxidations resulting from the lessened aëration of massive tissues, leaving a residue of malic acid, for example. *Castilleja*, however, presents the example of highly acid thin leaves, which become succulent under conditions similar to those which favor the transformation of polysaccharide to pentoses in other plants. Instead of acidity being a direct result of succulence, it is much more reasonable to conclude that high acid residues may be characteristic of plants which present a metabolic complex favorable to pentose formation and to the development of succulence under certain environic conditions.

[5] SPOEHR, H. A., The pentose sugars in plant metabolism. Plant World **20**:365. 1918.

[6] MACDOUGAL, D. T., and SPOEHR, H. A., The origination of xerophytism. Plant World **21**: 245–249. 1918.

COASTAL LABORATORY
CARMEL, CALIFORNIA

A CONIFEROUS SAND DUNE IN CAPE BRETON ISLAND

LEROY H. HARVEY

(WITH EIGHT FIGURES)

Nova Scotia has been called "the long wharf of Canada." Cape Breton Island, which is cut off from the mainland by the Gut of Canso, may be likened to its outermost pier. The island (fig. 1), which is about 100 miles long by 30 miles wide in its northern portion, extends in a northeasterly-southwesterly direction, restraining the waters of the Gulf of St. Lawrence on the west and separating them from the Atlantic Ocean on the east. The latitude of 47° north cuts the northern end of the island a few miles to the north of Aspy Bay, on whose shores the coniferous sand dune is located. Nova Scotia lies in the coniferous belt, which occupies the upland with the mixed hardwood formation occupying the most favorable situations along the narrow coastal strip. The interior is occupied by a vast expanse of wet and dry tundra-like formations, bordered by gnarled and twisted dwarf spruce, the entire vegetational aspect being decidedly coastal rather than alpine.[1]

The country is extremely rugged and the coastline jagged. Along the east coast is a narrow strip of sloping land, rarely a mile wide and often entirely lacking, which soon rises abruptly into an upland about 1000 ft. above sea level. In some places this upland plunges precipitously into the sea and the coast is very wild and bleak. This old Atlantic upland, which forms the backbone of the island, is the northern extension of the Piedmont Plateau. This upland has been cut during eons of erosion into deep gulches which extend far back into the central plateau. Down these gulches run swift and bowlder-bedded streams to the sea. At the mouth of these streams intervales are formed. Storm and tidal action have thrown shingle beaches and sand spits across the mouths of many of these reentrant bays (fig. 2). Upon one of these sand spits inclosing the South Pond of Aspy Bay is located the sand dune area which forms

[1] NICHOLS, G. E., The vegetation of Cape Breton Island, Nova Scotia. Trans. Conn. Acad. Sci. 22:251–467. 1918.

the subject of this study. It is the only sand dune noted on the island. The area is located some 5 miles from the crest of the upland and is fully exposed on its western side to the terrific northwest

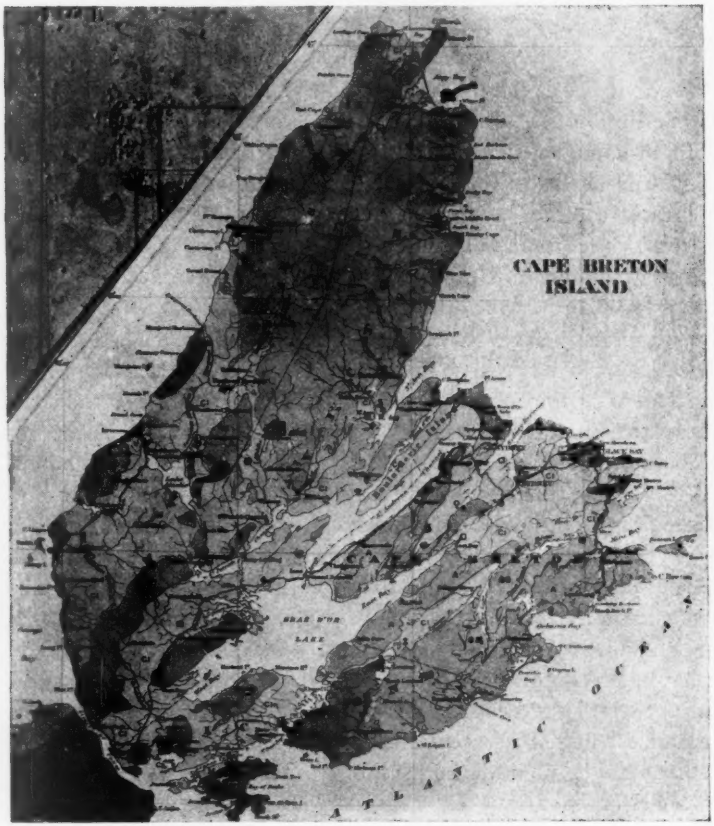


FIG. 1.—Cape Breton Island: Atlantic upland represented by dark shading; sloping marginal strip of lowland in light; Aspy Bay indicated by arrow.

winds, as well as to the cutting action of the outrunning tides from South Pond. The sand spit juts out to the southeast from a rocky upland reaching a total length of approximately $\frac{1}{2}$ mile, with a maximum width at the present time of less than 650 ft., and with a

minimum of less than 400 ft. But for the swift running tidal currents the spit would completely impound South Pond, extending across to the other headland; at best only a shallow and narrow channel now exists.

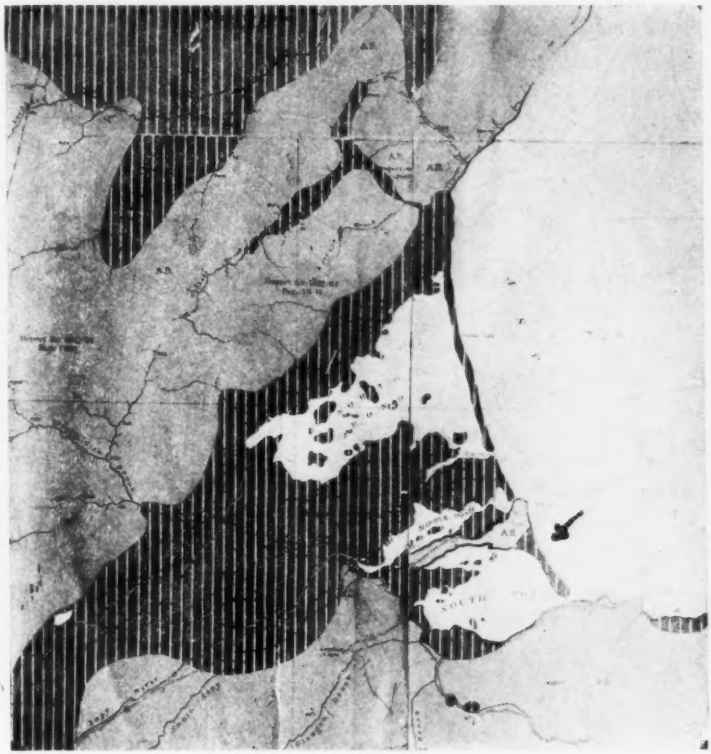


FIG. 2.—Aspy Bay region: sand spit on which sand dune is located marked by arrow; light shading indicates upland; dark shading indicates lowland.

The present condition of the area may be seen from fig. 3, which attempts to show the distribution of the existing plant associations. The dune complex, which is some 300 ft. wide, occupies less than one-half the length of the spit, being replaced in part at its southeastern extremity by a middle beach 400 ft. in width.

On the east the dune complex is fronted by a middle and lower beach, each with a width of approximately 100 ft. The lower

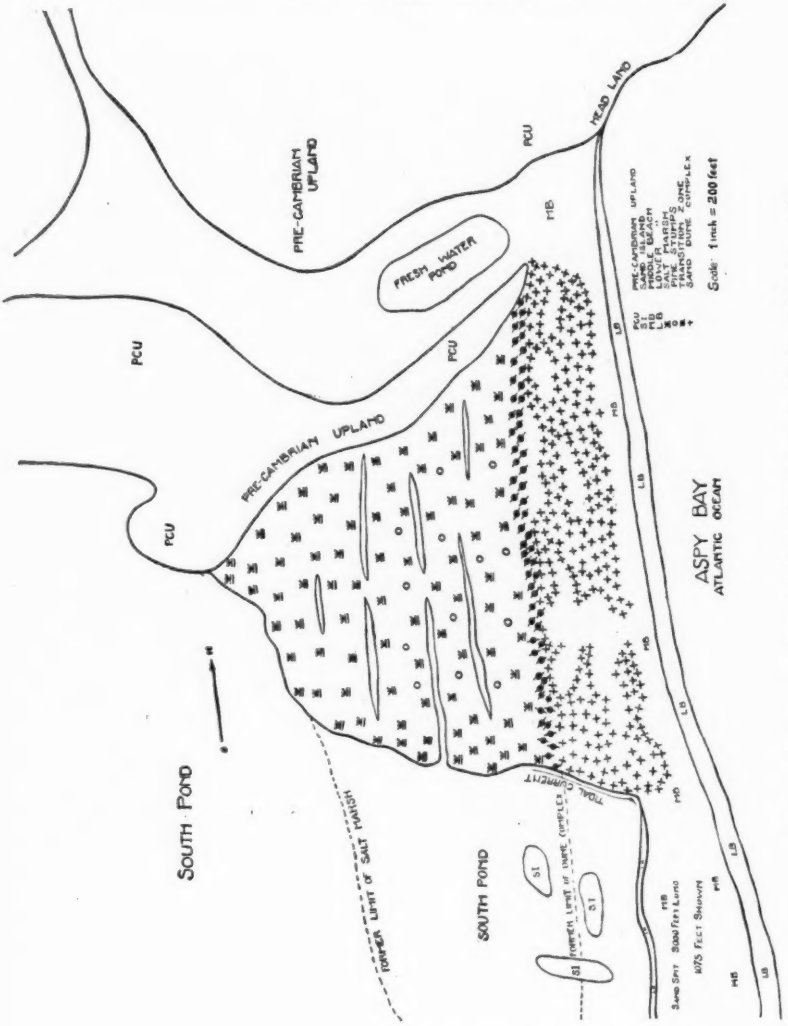


FIG. 3.—Diagram of present distribution of plant associations of dune complex

beach maintains its average width on the east side of the duneless end of the spit, where it has a gentle slope, but on its west side the

lower beach occupies a narrow steep margin only a few feet wide. To the immediate west of the spit in South Pond are several isolated sand islands (S.I.) mostly covered at high tide. To the west of the dune complex a transitional zone some 50 ft. wide separates it from an extensive salt marsh which is about a foot lower, and whose average width is estimated at 1200 ft. Several narrow salt water lagoons traverse this area in a north-south direction. Extending out into the marsh some 600 ft. along the eastern border is an area occupied by 10 or 15 old white pine stumps, approximately 100 years old, with well exposed roots, and standing in rows more or less parallel to the axis of the spit. The south end of the salt marsh and dune complex are suffering very active erosion under the daily outgoing tidal currents. High tides and occasional storms apparently sweep completely over the low duneless extremity of the spit, greatly augmenting this erosion. We may now consider each of these associations in greater detail.

Middle beach

The middle beach, which is extremely barren, is composed mostly of a fine sand, but shingle of a coarser nature is not wanting. The usual débris of the middle beach is encountered only to the east of the dune complex (fig. 4). The 3 principal plants are *Mertensia*[2] *maritima*, *Euphorbia polygonifolia*, and *Ammophila arenaria*, but all are exceedingly scattered. The only other species noted were *Glaux maritima*, *Lathyrus maritimus*, *Salsola Kali*, *Cakile edentula*, and these are represented only by occasional individuals.

Dune complex

The facies of the dune complex is *Picea canadensis* with occasional *Abies balsamea*. At the northern end the older trees were estimated at 75 years, while those at the southern, eroding end are scarcely 30–40 years of age. The southern (fig. 5) and eastern fringes of the complex include the highest dunes, which range from 3 to 15 ft. above sea level, and are generally margined by a narrow "grassy foredune" (fig. 6) with its precipitous slope oceanward. The sand binder is *Poa compressa*, a most unique condition. *Ammophila*, although scatteringly present, is of little importance in

[2] Nomenclature of GRAY'S *New Manual*, 7th ed.

FIG. 4.—Southeast end of dune complex, from east, showing middle beach, the *Picea-Abies* stand, and grassy foredune; South Pond and highland beyond is shown at extreme left; photograph by Dr. G. E. NICHOLS.

FIG. 5.—Southern and eroding end of dune complex, from south: South Pond and highland to west seen at left; middle beach is well shown; evidence of recent and rapid erosion plainly evident.

this respect. Associated species are *Euphorbia polygonifolia*, *Taraxacum*, *Iris versicolor*, *Rhus Toxicodendron*, *Rubus* sp., and *Ribes oxycanthoides*, all of which occur sporadically.

On the thickly wooded lea slope of these grassy dunes are found numerous woody and herbaceous species. The most prominent

FIG. 6.—Grassy foredune held by *Poa compressa*, from north: middle beach and naked spit seen to south; highland shown to south of South Pond (barely visible); photograph by Dr. G. E. NICHOLS.

species are *Iris versicolor*, *Campanula rotundifolia*, *Vaccinium Vitis-Idea*, *V. pennsylvanicum*, *Maianthemum canadense*, *Rhus Toxicodendron*, *Ribes*, and *Rubus*. The occurrence of these forms is sporadic.

In some places this outer range of dunes passes toward the west into low areas of considerable extent occupied by a unique association (fig. 3, blank areas in dune complex). Its aspect is grassy,

determined mainly by *Festuca rubra, Danthonia spicata, Agrostis maritima*, and *Panicum implicatum* in rather open formation. *Lechea intermedia, Vaccinium Vitis-Idaea, Potentilla tridentata, Fragaria virginiana terra-novae, Juniperus horizontalis, Empetrum nigrum, Barbula*, and a species of moss form more or less extensive mats. Other more scattered species are *Campanula rotundifolia, Euphrasia americana* (?), *Cerastium arvense* (?), *Solidago bicolor, Plantago maritima, Iris setosa* (?), *Veronica serpyllifolia, Arenaria lateriflora, Plantago major*, and several ruderals.

FIG. 7.—*Picea canadensis* showing layering; individual trees plainly seen in center of fig. 5.

A second and in some places a third series of much lower dunes is met in transect to the west. At the northern end of the complex practically all the white spruce is excessively infected with *Arceuthobium pusillum*, presenting the most remarkable development of witches' brooms it has ever been my privilege to see.

We have here a most remarkable physiographic condition of a dune moving seaward. The trees have mostly germinated at a lower level, and as the sand blows over the rounded top of the "grassy foredune" it forms a gentle lee slope to the west among these trees. As the trees are covered, abundant layering takes place, giving a long-lived and self-perpetuating stand (fig. 7). There

is some evidence, however, that germination actually takes place on these grassy dunes. Through layering and germination the complex slowly moves oceanward.

Salt marsh

The aspect of the salt marsh is determined by *Spartina glabra*, *S. patens*, and *Distichlis spicata*. *Juncus balticus littoralis* is very abundant along the drier margins. Other common species are *Salicornia europaea*, *Potentilla pacifica*, *Ranunculus Cymbalaria*;

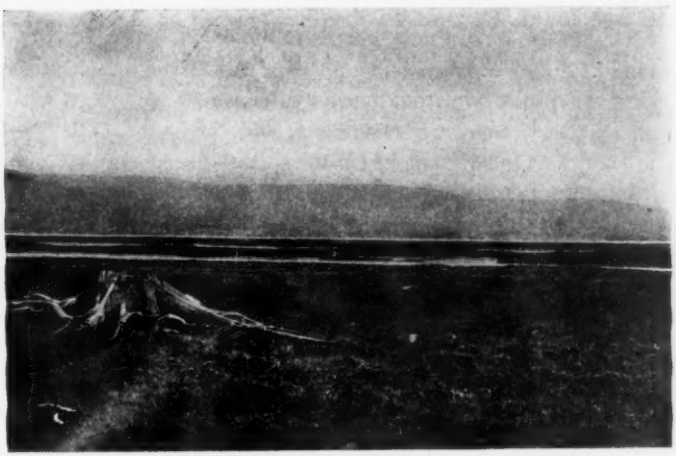


FIG. 8.—Salt marsh from eastern margin; *Pinus Strobus* stump in foreground; lagoons and South Pond in background; photograph by Dr. G. E. NICHOLS.

while *Vaucheria* and *Cladophora* occur in great mats on the margins of pools. The most striking feature, however, is the presence of numerous white pine stumps (fig. 8), remnants of a lumbering operation, whose distribution simulates rows parallel to the axis of the spit extending from the eastern shore out into the marsh to a distance of several hundred feet. The roots of these stumps are well exposed. It is evident that they must have germinated upon land possibly a foot or more higher than this, somewhat over a century ago. It is also evident that the salt marsh has encroached from the west and is moving eastward. Coastal elevation or denudation

could account for this encroachment, and I believe the latter more probable. The erosive force might well have been wind, acting subsequent to the removal of the white pine forest which evidently existed here. High tidal action has undoubtedly cooperated in the removal of the upper part of what appears to have been an extensive sand plain, as indicated by the north and south extensions of the lagoons.

Restoration of original condition

Within a century it seems probable that an area somewhat more extensive to the south than that now occupied by the salt marsh, and lying in the lee of the highlands to the north, was covered with a stand of *Pinus Strobus*. The present area of the dune complex, lacking this protection from marine influences, was covered with a stand of *Picea canadensis* and *Abies*, and this stand extended in its full width to the present end of the spit. It seems probable that the dune complex is a relatively recent phenomenon, developing subsequent to the removal of the stand of white pine. At about this same time the channel of the tidal current was changed and began cutting at the south end of the spit, eroding the *Picea* stand (fig. 5). According to a native, about one-third of this erosion has been accomplished in the last 35 years, or at the rate of 25 ft. per annum. If this rate has been approximately constant, the *Picea* stand was intact within a century to the end of the spit, which now lies bare for about one-half mile (fig. 3).

Summary

It is the purpose of this paper to put on record several facts of ecological interest: (1) a coniferous sand dune with *Picea canadensis* as its facies located at the latitude of 47° north; (2) *Poa compressa* as a sand binder; (3) abundant layering in *Picea canadensis* and *Abies balsamea;* (4) the anomalous condition of a sand dune moving seaward; (5) a phenomenal development of *Arceuthobium pusillum* on *Picea canadensis;* (6) the decisive value of ecological data in the interpretation of physiographic phenomena.

WESTERN STATE NORMAL SCHOOL
KALAMAZOO, MICH.

EMBRYO SAC AND EMBRYO OF PENTSTEMON SECUNDIFLORUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 248

ARTHUR T. EVANS

(WITH PLATE XII)

Two genera of the Scrophulariaceae were the first plants in which the development of the embryo sac and the embryo were correctly investigated. In 1851 HOFMEISTER (**8**), working on *Lathraea squamaria* and *Pedicularis sylvatica*, proved that the embryo was formed as a result of the fertilization of the egg, and not from the end of the pollen tube as was believed by SCHLEIDEN and his followers. DEECKE (**5**) in 1855 reinvestigated *Pedicularis sylvatica*. He insisted that HOFMEISTER was wrong and that the embryo really did develop from the end of the pollen tube. He was supported in his assertions by SCHACHT (**11**). Later, however, HOFMEISTER (**9**) proved that what DEECKE really saw was the proembryo. In his paper on *Lathraea* and *Pedicularis*, HOFMEISTER discusses the beginning of the endosperm and the haustoria. No further work of importance was done upon the Scrophulariaceae until 1874, when CHATIN (**3**) studied the development of the ovule and the seeds in a number of genera. Four years later VESQUE (**14**) worked on the embryo sac of a number of families, among which were included several of the Scrophulariaceae. Even as late as his publication of this paper VESQUE believed that SCHLEIDEN'S theory of the formation of the embryo was correct, and criticized HOFMEISTER'S interpretation as inaccurate.

One of the best contributions to our knowledge of the embryo sac situation in this family is by BALICKA-IWANOWSKA (**2**) in 1899. The account includes a study of a number of families of the Sympetalae, but deals especially with several genera of the Scrophulariaceae, particularly taking up the question of nutrition in the embryo sac. The haustoria are believed to have an absorptive power, and thus conduct nourishment into the embryo sac, the

conclusions being based on the fact that the haustoria are always found in contact with parts which are well supplied with nourishment. Miss BALICKA-IWANOWSKA disagrees with HEGELMAIER (7) as to the function of the tapetum. HEGELMAIER believed it to act as a protective covering, while the former seemed to prove that it serves to pass nutritive substances on to the embryo sac, and that it possibly has a digestive function, since the cells of the integument adjoining it are found constantly breaking down. In 1906 SCHMID (12) investigated numerous species of the Scrophulariaceae. He discusses the formation of the embryo sac, fertilization, endosperm formation, and the development of the haustoria. He has done very little with the development of the embryo. In 1915 Miss MITCHELL (10) investigated the embryo sac and the embryo in *Striga lutea*, a semi-parasitic plant found in South Africa. In this form she has noted the lack of a tapetal layer.

The material for this study was collected near Boulder, Colorado, where the species is abundant. The indeterminate inflorescence affords flowers in all stages of development on the same plant. Such material was killed in chrom-acetic acid, cut in paraffin, and stained in various stains, safranin-gentian violet proving the best. Longitudinal sections 10 μ thick proved quite satisfactory for study.

The writer is indebted to Dr. FRANCIS RAMALEY of the University of Colorado for advice during the early stages of the work, and especially to Dr. CHARLES J. CHAMBERLAIN of this laboratory, under whose direction the work was completed, for his kindly aid and criticism.

Ovary and embryo sac

The ovary of *Pentstemon secundiflorus* Benth. is of the ordinary bilocular scrophulariaceous type, with the partition somewhat swollen in the median line forming the placenta, which bears the numerous crowded anatropous ovules. Longitudinal sections of such an ovary at right angles to the partition afford a large number of ovules in each section for study. Sections of very young ovaries show the ovules beginning as slight swellings of the placenta. The megaspore mother cell is not distinguishable in such an early stage, becoming apparent only after the beginning of integument forma-

tion. It appears as a single enlarged and darker staining subepidermal cell, which functions directly. Growth is quite rapid and the cell soon becomes elongated. It is surrounded by a single layered nucellus. The single integument forms along the sides of the nucellus and soon surrounds it. A short time before the integument has completely surrounded the ovule the megaspore mother-cell has entered synapsis (fig. 1). Miss MITCHELL has estimated that about 10 per cent of the ovules of *Striga lutea* have reached synapsis at the same time. This percentage may safely be placed much higher for *P. secundiflorus*, probably more than 75 per cent of the ovules of a single ovary showing the same stage of development.

By the time the nucleus of the megaspore mother-cell has entered synapsis the young ovule is rapidly assuming its anatropous form, which is reached by the time the reduction divisions are completed. The first reduction division occurs about the time the integument has surrounded the ovule completely. This division is soon followed by the second, forming the row of 4 megaspores. Either the third or the fourth megaspore of the row may function in forming the embryo sac (figs. 2, 3). The other 3 disintegrate rapidly and become crushed by the growth of the one functioning.

The megaspore which functions increases rapidly in size, the micropylar end becoming bulbous while the chalazal end remains narrowed (fig. 4). The chalazal end, however, lengthens rapidly until it is 2–4 times as long as the bulbous portion. This growth carries it to a point in contact with the end of the vascular system. The nucellus early disappears, but by the time the embryo sac is formed another nutritive layer, the tapetum, has formed from the integument. During the growth of the embryo sac the single nucleus by 3 divisions has formed the 8-nucleate sac. The rapid growth of the sac causes the protoplasm to be much vacuolated.

In the earliest stage of the 8-nucleate sac 4 nuclei are found grouped at each end (fig. 4). Soon, however, a nucleus from each end migrates toward the opposite end. Eventually they meet and form the polar fusion nucleus (fig. 5). BALICKA-IWANOWSKA and SCHMID have commented upon the place of this fusion. The former says that it occurs near the middle of the sac, while the

latter finds that it may occur anywhere in the sac of the Scrophulariaceae studied by him. In *P. secundiflorus* polar fusion was found to take place anywhere, seeming to be more a matter of chance than any regulated procedure. Regardless of where polar fusion takes place, the polar fusion nucleus is always found in the bulbous micropylar end of the sac at the time of fertilization (figs. 6, 7). It is here that the triple fusion is completed. By the time polar fusion is completed the egg apparatus is well formed and the antipodals have begun to disintegrate. In only one case were the antipodals observed to form anything resembling cell walls. The mature embryo sac (fig. 5) is one of the commonest stages of the sac to be found. This is probably due to failure to pollinate at once. A short period of inactivity always seems to occur.

The mature embryo sac is interesting in that it is always well filled with starch (figs. 6, 7). As soon as the megaspore begins its development into the embryo sac, traces of starch are to be found in it, although it is not until the embryo sac is well matured that large quantities of starch are present. Very often the adjacent tissues contain much starch also. After fertilization, when the endosperm and the embryo begin to develop, the starch in the sac disappears entirely. Many of the grains found in the sac are large, reaching 30 μ or more. Although starch is to be found in either end of the embryo sac it is always much more abundant in the micropylar end. SCHMID has found this to be true also.

False polyembryony

The fusion of 2 ovules appears to be a much more uncommon occurrence than the formation of 2 or more embryo sacs in a single ovule. Miss MITCHELL discusses a single case which she found in *Striga lutea*. The only other plants in which it has been reported are *Pyrus Malus*, *Loranthus europaeus*, and *Viscum album* (4). In the course of this study several cases in which 2 ovules had fused were noted. In some the fusion was quite complete, in others the ovule could be seen to be double. The presence of 2 micropyles as well as integumentary tissue between the 2 embryo sacs indicated that 2 ovules had fused. In one instance noted the

egg apparatus had formed and polar fusion had occurred in both embryo sacs. False polyembryony seems to be quite common in this species.

Fertilization

In several cases the pollen tube with the tube nucleus and the 2 sperms were observed. While in the pollen tube the sperms are more or less capsule-shaped, but after reaching the embryo sac they become quite spherical. The pollen tube seems always to enter the embryo sac a little to one side, its entrance usually destroying one of the synergids, the other synergid disappearing soon afterward.

The sperms are readily distinguished from the egg nucleus and the polar fusion nucleus on account of their much smaller size. Fertilization of the egg and the triple fusion always occur in the micropylar end of the sac and in a normal manner. Both fusions occur at approximately the same time.

Several cases of double fertilization were observed (fig. 7). Previously double fertilization has been announced as occurring in *Digitalis purpurea*, *Linaria vulgaris*, *Melampyrum sylvaticum*, *Lathraea squamaria*, *Pedicularis foliosa*, and *Striga lutea* of the Scrophulariaceae. This adds *Pentstemon secundiflorus* to the list.

Formation of endosperm

Without resting after the fusion with the sperm, the endosperm nucleus by a series of divisions forms a large number of nuclei, which migrate to the chalazal end of the sac and there become peripherally placed. Simultaneous with the formation of the free endosperm nuclei the narrowed end of the sac begins to increase in size very rapidly, so that it soon surpasses the micropylar end in diameter (fig. 8). By the time the first endosperm walls have formed this end of the sac is much the larger. During all this increase in size a certain restricted area between the 2 ends remains very narrow, so that the embryo sac comes to be dumb-bell-shaped, with the chalazal end the larger. Endosperm walls continue to form in this end until the whole is completely filled (figs. 8, 10). Although endosperm nuclei are occasionally found in the micropylar end of the sac, no cell walls were observed to form. During

endosperm formation the tapetum appears to be very active. Integumentary cells in contact with it are broken down and the tapetal cells are always filled with a dense protoplasm.

Haustoria

With the formation of the endosperm 2 large haustoria are formed: one in the neck which connects the micropylar and chalazal ends of the sac (fig. 8), the other as an outgrowth from the chalazal end of the sac (fig. 9). The former is formed by the growth of 2 endosperm cells forward through the narrowed neck and just into the micropylar end of the sac where growth stops. In the case of the chalazal haustorium there is an outgrowth of the sac in the region not covered by the tapetum. Into this bulbous pocket 4 endosperm cells grow. This brings the endosperm cells well into connection with the vascular tissue, the cells of which are gorged with nutritive material. The protoplasm of each haustorium is very dense. The cells of the chalazal haustorium are binucleate.

The active tapetal layer covers only the chalazal end of the sac (fig. 8), ending abruptly at its junction with the micropylar end. SCHMID found that the tapetum might cover all of the embryo sac or only part of it as in *P. secundiflorus*. The latter condition seems the more common occurrence. Miss MITCHELL found that no tapetum is formed in *Striga lutea*. She believes that this may be accounted for by the semi-parasitic habit of the plant.

Development of embryo

After fertilization the egg rests for a time, often even until endosperm cell walls have begun to form. It then divides, the first division being at right angles to the axis of the embryo sac. The segment nearest to the micropyle forms the suspensor, the other forming the embryo. By a series of divisions, coupled with rapid growth, the suspensor is transformed into a bulbous basal portion, and a number of smaller narrowed cells which lengthen rapidly in such a manner as to push the 1-celled embryo through the micropylar end of the sac (fig. 8) and into the center of the endosperm beyond (fig. 10). It is usually pushed from one-third to one-half

of the way through the endosperm, where further progress is probably stopped by the division and growth of the embryo. The first 2 divisions of the embryo are at right angles to each other and in the plane of the long axis of the sac. The next division is at right angles to the first two and forms the 8-celled stage of the embryo. The 16-celled stage is formed by the periclinal division of the cells of the octant. The further division of the embryo was not followed.

After the embryo becomes imbedded in the endosperm the micropylar end of the sac, together with the suspensor, collapse and disappear. Their disappearance is accounted for by the pressure within the ovule, due to the increase in amount of endosperm which eventually comes to occupy all the space inside the seed coat.

Discussion

In the formation of the embryo sac of *P. secundiflorus* there is nothing strikingly different from that of other species of this family which have been studied, but the shape of the mature embryo sac is peculiar. The very bulbous micropylar end, with the long, narrowed chalazal end, gives the whole embryo sac a club-shaped appearance. The chalazal part of the embryo sac is never more than half as wide as the micropylar end at the time of fertilization. The drawings of the embryo sac of other Scrophulariaceae by BALICKA-IWANOWSKA, SCHMID, and MITCHELL show that there is a tendency toward this shape of embryo sac in the family, but none of those drawn are so striking in shape as that of the species under consideration. The distance between the end of the embryo sac and the end of the vascular system is at first marked. As the sac later derives a large part of its nourishment through the vascular system, this may account for the necessity of lengthening the sac until the end comes in contact with this source of food supply.

During the development of the embryo sac traces of starch can be seen within it, and in all cases, by the time fertilization occurs, large quantities of starch are present. Often it is so abundant that the nuclei within the sac are partially or entirely obscured. By the time the embryo has reached the endosperm the starch has all disappeared.

D'HUBERT (6) has made a study of fleshy plants with regard to the formation of starch in the embryo sac. He finds that starch is always present in the sacs of fleshy plants such as the Cactaceae, Mesembrianthaceae, Crassulaceae, Portulacaceae, etc. He has also found, however, that some non-fleshy plants show starch in the embryo sac. According to D'HUBERT this latter case seems to be the exception rather than the rule, and he believes that there is a relationship between the fleshiness of the plant and starch in the embryo sac due to the slowness of the phenomena before fertilization. This, however, receives very little attention from him; nevertheless it seems the more plausible theory. *P. secundiflorus* is not a fleshy plant, but, judging from the drawings which D'HUBERT has made of several fleshy plants, it has more starch in its embryo sac than any of those figured. It appears that while there is activity in the embryo sac very little if any starch is stored up. As soon as the embryo sac matures and becomes inactive just before fertilization, possibly due to delay in pollination, the stream of nourishment which has been coming in cannot be checked suddenly but keeps passing more and more nutrition into the inactive sac, where it is stored in the form of starch. Such a conclusion seems to be substantiated by the fact that activity in the sac brought about by fertilization soon reduces the amount of stored-up starch. BALICKA-IWANOWSKA (2) has also investigated the deposition of starch in the embryo sacs of several plants, and has concluded that starch is only found in the embryo sac when the tapetum is cutinized. This does not seem to be the case in *P. secundiflorus*, however, as the tapetum is undoubtedly not cutinized. Moreover, it covers only half of the embryo sac, as has been explained before. SCHMID (12) has found starch present in the integuments as well as the embryo sacs of a number of the Scrophulariaceae. He states that the starch is found throughout the embryo sac, but that sooner or later it is all translocated to the micropylar end, "wo die lebhaftesten Teilungen stattfinden."

The function of the tapetum seems to be one of nutrition, as has been suggested by BALICKA-IWANOWSKA (2). That it may have a protective function, as has been suggested by HEGELMAIER (7), seems rather doubtful. This seems all the more questionable

when one considers that it covers only the chalazal end of the sac in a number of species. Surely the micropylar end would be as much in need of protection. In *P. secundiflorus* the integumentary cells border on the micropylar end of the sac.

The two haustoria function in passing nourishment to the endosperm cells which are farther from the supply of food. By the time the embryo has reached the endosperm the micropylar haustorium becomes inactive and is lost. The chalazal one, however, functions until the endosperm is formed. The nuclei in this haustorium are very pronounced. On account of the large size and seeming activity of haustorial nuclei some authors have attributed to them a considerable rôle in nutrition. BALICKA-IWANOWSKA (2) has always found them near the point where nutrition is most abundant. In this work a similar tendency was noted.

The growth of the suspensor in such a manner as to push the proembryo through the micropylar end of the embryo sac and to imbed it in the endosperm is rather unique. SHARP (13) in his study on *Physostegia* has recorded a similar situation, but the method of endosperm formation in *Physostegia* and *Pentstemon* is different entirely.

During the growth of the suspensor which imbeds the embryo in the endosperm, nutrition is derived from the starch stored up in the micropylar end of the sac.

Summary

1. The embryo sac is developed from a single megaspore. Its antipodals disorganize early. The micropylar end becomes bulbous, while the chalazal end becomes long and narrow and is covered by a distinct tapetum.

2. The mature embryo sac is found to be constantly gorged with starch, due to the non-utilization of the nutritive materials which pass into the sac at a time of inactivity just before fertilization.

3. The endosperm nucleus immediately divides and free nuclei migrate into the chalazal end of the sac, where wall formation begins. The proembryo is pushed into this endosperm by an

extreme growth of the suspensor. The micropylar end of the sac disintegrates.

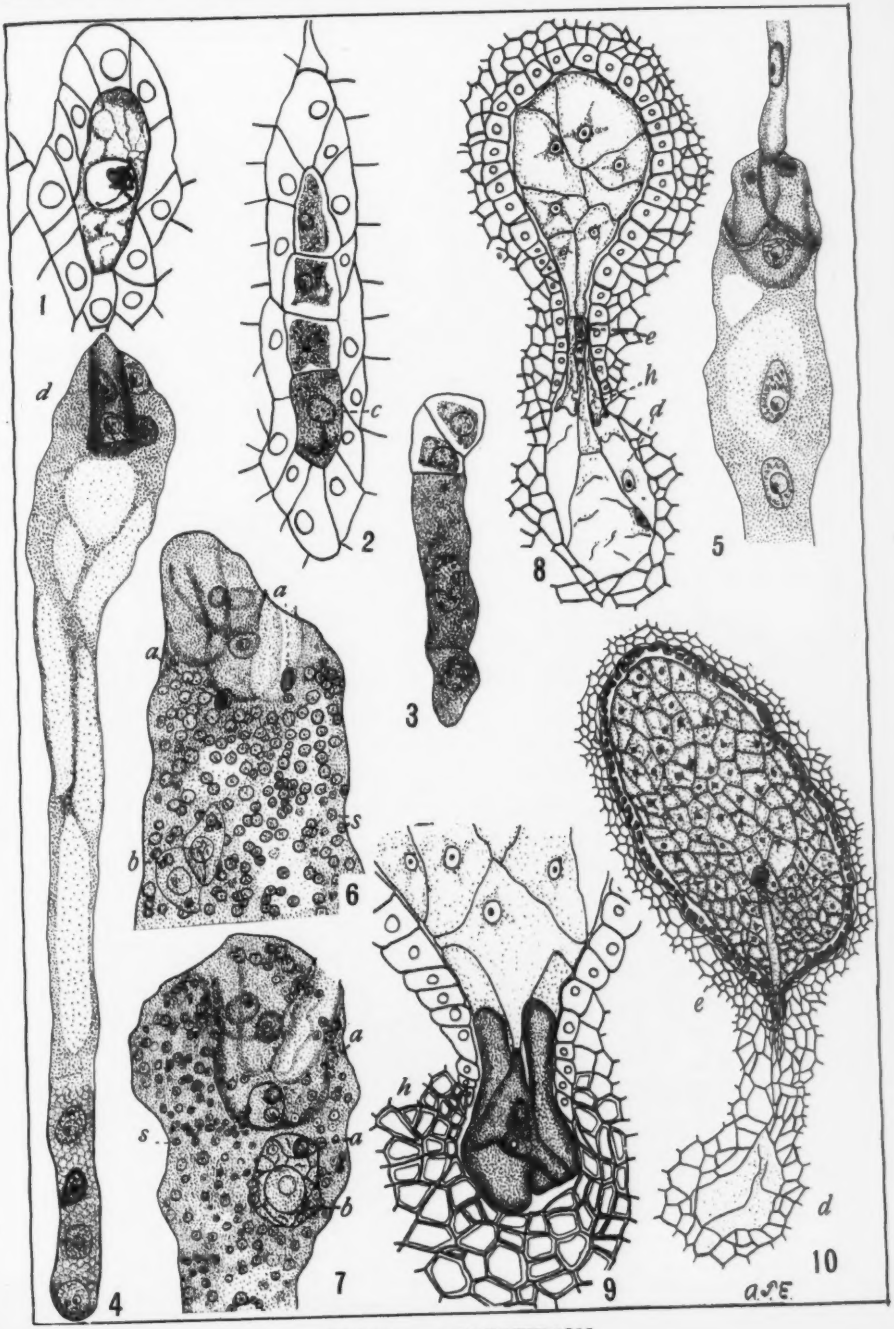
4. Two haustoria are formed, the micropylar by the growth of endosperm cells from the chalazal end into the micropylar end, and the chalazal by a growth of endosperm cells from the chalazal end out into the vascular system. The cells of the latter haustorium are binucleate.

5. *False polyembryony occurs rather commonly in this species.*

PURDUE AGRICULTURAL EXPERIMENT STATION
LAFAYETTE, IND.

LITERATURE CITED

1. BACHMANN, E. TH., Darstellung der Entwicklungsgeschichte und der Baues der Samenschale der Scrophulariaceen. Nova Acta Leop. **43**:1–177. *pls. 1–4.* 1888.
2. BALICKA-IWANOWSKA, G., Contribution a l'étude du sac embryonnaire chez certaines Gamopetales. Flora **86**:47–71. *pls. 3–8.* 1899.
3. CHATIN, J., Etudes sur la developpement de l'ovule et de la graine. Ann. Sci. Nat. Bot. V. **19**:1–107. *pls. 1–8.* 1874 .
4. COULTER, J. M., and CHAMBERLAIN, C. J., Morphology of angiosperms. New York. 1903.
5. DEECKE, TH., Nouvelles recherches sur la developpement du *Pedicularis sylvatica.* Ann. Sci. Nat. Bot. IV. **4**:48–63. 1855.
6. D'HUBERT, M. E., Recherches sur le sac embryonnaire des plantes grasses. Ann. Sci. Nat. Bot. VIII. **2**:37–128. *pls. 1, 2.* 1896.
7. HEGELMAIER, FR., Über den Keimsack einiger Compositen dessen Umhullung. Bot. Zeit. **47**:805–812, 821–826, 837–842. 1889.
8. HOFMEISTER, W., Zur Entwicklungsgeschichte des Embryo der Personaten. Neue Reihe. **9**:449–457. *pls. 8, 9.* 1851.
9. ———, Neuere Beobachtungen über die Embryobildung der Phanerogamen. Pringsh. Jahrb. **1**:1858.
10. MITCHELL, MARGARET, Embryo sac and embryo of *Striga lutea.* BOT. GAZ. **59**:124–135. *pls. 8, 9.* 1915.
11. SCHACHT, H., Sur l'origine de l'embryon végetale. Ann. Sci. Nat. Bot. IV. **3**:180–208. *pls. 7, 8.* 1855.
12. SCHMID, E., Beiträge zur Entwicklungsgeschichte der Scrophulariaceen. Zurich. 1906.
13. SHARP, L. W., The embryo sac of *Physostegia.* BOT. GAZ. **52**:218–223. *pls. 6, 7.* 1911.
14. VESQUE, M. J., Developpment de sac embryonnaire. Ann. Sci. Nat. Bot. VI. **8**:261–390. *pls. 12–21.* 1879.



EVANS on PENTSTEMON

EXPLANATION OF PLATE XII

All the drawings were made at table level with the aid of an Abbé camera lucida. The labels are: *a*, sperm; *b*, polar fusion nucleus; *c*, megaspore; *d*, micropylar end of embryo sac; *e*, embryo; *h*, haustorium; *s*, starch; *t*, tapetum.

FIG. 1.—Synapsis in the megaspore mother-cell; ×880.

FIG. 2.—Row of 4 megaspores showing fourth developing into embryo sac; ×880.

FIG. 3.—Row of 4 megaspores showing third developing into embryo sac; ×880.

FIG. 4.—An 8-nucleate embryo sac before maturity, showing characteristic shape of sac and egg apparatus beginning to form; ×880.

FIG. 5.—Micropylar end of mature embryo sac showing egg apparatus well formed and migration of polar fusion nuclei; ×460.

FIG. 6.—Micropylar end of mature embryo sac showing egg apparatus, entrance of pollen tube, 2 sperms, polar fusion, and starch; ×880.

FIG. 7.—Mature embryo sac showing double fertilization; ×880.

FIG. 8.—Stage showing growth of suspensor pushing young embryo between micropylar haustoria which have already begun to disintegrate; section a little to one side of chalazal haustorium; tapetum present; ×460.

FIG. 9.—Stage showing binucleate chalazal haustorium; ×880.

FIG. 10.—Late stage of endosperm formation showing embryo imbedded; micropylar end of sac already beginning to collapse; ×460.

BRIEFER ARTICLES

DEPRESSED SEGMENTS OF OAK STEMS

(WITH FOUR FIGURES)

In a recent paper, Miss LANGDON[1] questions certain statements of the writer[2] in regard to the deeply depressed or sunken segments which occur commonly in stems of *Quercus*. She states (p. 321):

> From observations of transverse sections of twigs from *Quercus alba*, *Q. bicolor*, and *Q. macrocarpa* I find that there is evidence of retardation in growth of the tissues in the immediate vicinity of the wide rays, especially noticeable in the marked dipping in of the annual rings where they cross the large rays. However, aside from a few extreme cases, this checking influence of the wide foliar rays does not explain the 5 conspicuous depressions so characteristic of the wood of *Quercus*.

In discussing the topographical features of the stem of the oak, it is essential to distinguish between two different factors which have modifying effects upon the outline of the secondary xylem. I refer to the arrangement of the primary elements and the development of multiseriate rays. The effects of these two factors, or complexes of factors, may be studied most satisfactorily in plants where they occur independently. For example, in *Castanea dentata* and *Populus balsamifera*, which normally have only uniseriate rays, the primary elements have the stellate arrangement that is characteristic of *Quercus*. In the internodes of normal stems of these plants, the first formed secondary elements form a layer of undulating or stellate outline, but at the end of two or three growing seasons, frequently earlier, the outer periphery of the secondary xylem tends to be circular (fig. 1). In other words, the early lobed or stellate form of the cambium soon becomes evanescent, and its effects upon the shape of the stele are quite transient.

The modifying influences of the second set of factors, acting independently of the first, may be seen quite clearly in the stems of certain

[1] LANGDON, LADEMA M., The ray system of *Quercus alba*. BOT. GAZ. **65**: 313–323. 1918.

[2] BAILEY, I. W., The relation of the leaf trace to the formation of compound rays in the lower dicotyledons. Ann. Botany **25**:225–241. 1911.

species of *Amphilophium*. Fig. 2 illustrates a cross-section of the stem of one of these plants. There are 4 pairs of approximated multiseriate rays in the fourth growth layer. The narrow segments of xylem between these wide rays are deeply depressed below the general outline of the stem. They obviously are not correlated with a lobed or stellate arrangement of the primary elements, but are due to differences in the number of xylem elements formed by different arcs of the cambium during the fourth growing season.

That the deeply depressed segments which occur commonly in oak stems, having 2–10 or more growth layers, are correlated with the presence of pairs of approximated multiseriate rays rather than the

FIG. 1

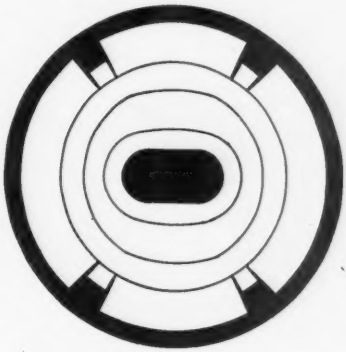


FIG. 2

stellate arrangement of the primary elements, is indicated not only by a comparative study of the stems of various arborescent dicotyledons, but also by numerous facts in the anatomy of the genus *Quercus*. From different species of oaks and from plants grown under different environmental or experimental conditions, it is possible to secure a series of stems showing various stages in the disintegration and disappearance of multiseriate rays.[3] The segments are most deeply depressed in specimens in which the pairs of multiseriate rays are most conspicuously developed (fig. 3). On the other hand, where the pairs of wide rays or their vestiges ("aggregate rays," etc.) are entirely absent, the stellate form of the early cambium, which may be conspicuous during the first growing season or two, quickly becomes circular, as in *Castanea* and *Populus*. Where there

[3] BAILEY, I. W., and SINNOTT, E. W., Anatomical evidences of reduction in certain of the Amentiferae. BOT. GAZ. **58**:36–60. 1914.

is a marked retardation in the development of the pairs of rays the appearance of the deeply depressed segments is coincident with that of the rays (fig. 4). Particularly significant are those stems in which one ray of a pair fails to develop. Under these circumstances, the narrow segment of xylem tends to be unsymmetrically depressed (fig. 4). Furthermore, the fact that depressed segments may occur between pairs of rays, which are opposite the projecting lobes of the pith (fig. 3), and between approximated "secondary" rays, suggests that the stellate outline of the early cambium is not an indispensible factor in the production of the sunken wedges of xylem in oak stems.

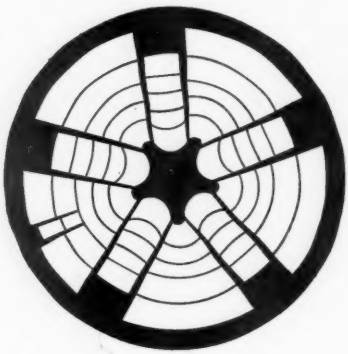


FIG. 3

FIG. 4

Miss LANGDON offers a physiological explanation for the stellate form of the stele in young twigs (p. 321):

> Since the principal function of the xylem is the conduction of water from the soil to the outer parts of the plant, it is obvious that the maximum upward movement of solutions in the stem would be through the tracheidal tissues and vessels in direct line with the leaf traces. This would cause an acceleration in growth and the consequent outward projection of those five regions of the woody cylinder associated with leaf traces, while the neighboring conducting tissues, namely, the so-called depressions from which the main conducting streams had been diverted to the petioles of the leaves, would fail to maintain their normal rate of growth.

It is to be emphasized, in this connection, that the projecting wedges of the first annual rings of *Castanea*, *Populus*, and *Quercus*, when devoid of wide rays, are not due to an acceleration of growth. This can readily be determined by measuring the depth of the convex and concave arcs of

xylem in one or two year old stems. The average depth of the latter almost always equals and usually exceeds that of the former (fig. 1), indicating conclusively that there is no growth acceleration in the convex arcs of the cambium which form the projecting wedges. As has been indicated by the writer, the undulating outline of the first formed secondary xylem is due to the stellate arrangement of the primary elements, and consequently the stellate outline of the first formed cambium. However, this originally lobed cambium rapidly takes on a circular outline, owing to the slower growth of its convex projecting arcs, except in stems which have a hereditary tendency for the formation of pairs of approximated multiseriate rays.—I. W. BAILEY, *Bussey Institution, Jamaica Plain, Mass.*

IMPORTANCE OF EPIDERMAL COVERINGS[1]

(WITH TWO FIGURES)

In making tests of the relative resistance of some herbaceous plants to freezing, it was observed that inoculation from ice formed on the leaf surface was a factor of great importance in determining the temperature at which ice formation occurred in the leaf tissue. In testing cabbages it was observed that the greatest undercooling of the tissue below its freezing point occurred in those plants which had the greatest amount of "bloom" on the leaf surface. Plants well covered by wax could be maintained for hours at a temperature 5°C. below their freezing point without the formation of ice in the tissues. Similar conditions were found to occur in the common *Cineraria* and other such plants which are densely covered with a mat of epidermal hairs. This condition suggested that inoculation of the undercooled leaf tissue by ice formed on the leaf surface was an important factor in frost resistance. The object of this study was to determine the amount of undercooling which can occur in such tissues, and the importance of the epidermal coverings in preventing surface inoculation of the undercooled tissues.

The thermoelectric method was used to measure temperatures, since this method allows one to determine the temperature inside rather thin leaves. A copper-constantan couple of no. 40 B. and S. gauge which had a thermal coefficient of 3.33 millivolts per degree Centigrade was used. Using such a couple the delicacy of the potentiometer arrangement determines the accuracy of the temperature measurement. Although with the arrangement used much smaller changes could be

[1] Published by permission of the Secretary of Agriculture.

determined, measurements to 0.1°C. or less were found to be sufficiently accurate for this work.

Fig. 1 shows the arrangement for temperature measurement as well as the means for securing undercooling of the tissues. Directions for constructing the thermal junctions are to be found in the publications of WHITE from the Carnegie Institution of Washington.[2] The constant temperature junction of the thermocouple was placed in a thin-walled tube filled with oil and immersed in a slush of clean snow in distilled water contained in a Dewar beaker. It was found that the temperature of this junction could be kept constant for a long time within a few thousandths of a degree Centigrade, as shown by a standard Beckmann thermometer. In calibrating the couple, the junction to be placed within the leaf was placed in a Dewar beaker similar to the former arrangement, with solutions having freezing points a few degrees below zero.

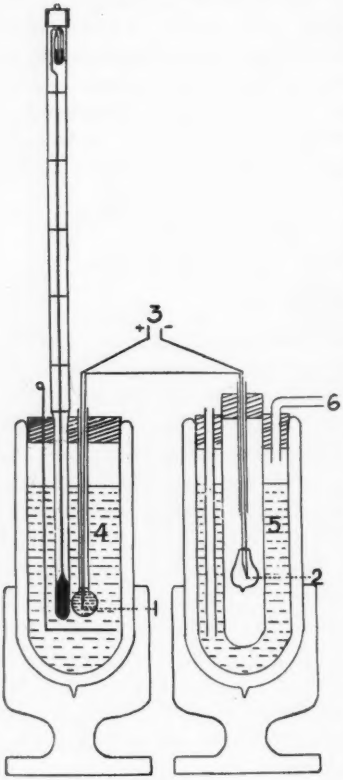


FIG. 1

A number of tissues were tested with this apparatus, including carnation stem, cabbage leaf, *Echeveria* leaves, *Cineraria* petiole, tomato petiole, and others. In general the results show that plants with heavy epidermal coverings of wax or trichomes can undergo a much greater undercooling than such plants as tomatoes, in which the epidermal walls are thin and non-resistant.

[2] WHITE, W. P., The thermoelement as a precision thermometer. Physical Review **31**:135. 1910.

———, The constancy of thermoelements. Physical Review **23**:449. 1906.

WHITE, W. P., DICKINSON, H. C., and MUELLER, E. I. The calibration of copper-constantan thermoelements. Physical Review **31**:159. 1910.

To make certain that all of the samples should have ice formed on the surface, a small drop of water was placed on each. To test the effect of the epidermal covering in preventing inoculation, samples of the same material were cooled with the coverings intact, and also after they had been removed. Precautions were taken to prevent the inoculation of the tissue from juices exuded at cut surfaces. In all cases the cut surfaces were dried and covered with vaseline, which procedure was found to prevent such an inoculation of the tissue. In all the tests it was

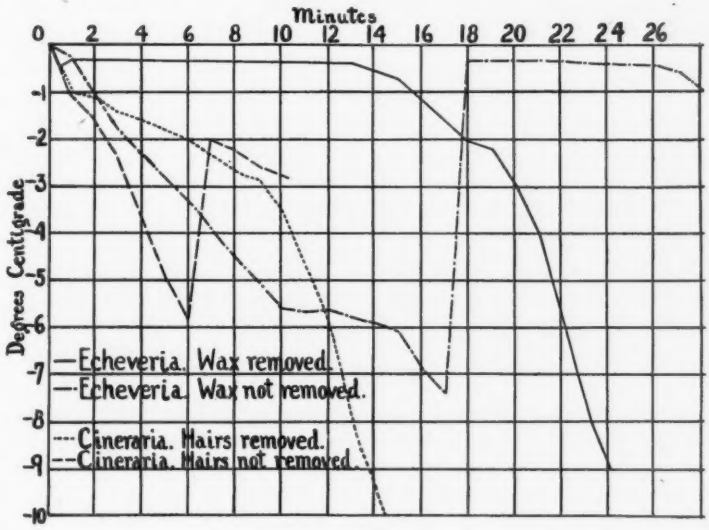

Minutes
0 2 4 6 8 10 12 14 16 18 20 22 24 26
-1 -2 -3 -4 -5 -6 -7 -8 -9 -10
Degrees Centigrade
—Echeveria. Wax removed.
—Echeveria. Wax not removed.
---Cineraria. Hairs removed.
--Cineraria. Hairs not removed.

FIG. 2

found that when thick epidermal coverings were unbroken, undercooling occurred, while in those cases where they were removed but little undercooling occurred.

Fig. 2 shows typical cooling curves, indicating the importance of the epidermal coverings in preventing ice formation within the tissue. It appears that the tissue without the protective covering is unable to undergo any great amount of undercooling. This depends in part upon the amount of shaking of the tissue. The osmotic concentration or the presence of colloidal substances in solution in the cell sap are of relatively minor importance in determining the undercooling.

It has been observed during spring frosts that in blooming apricots those buds which have opened and are turned upward to collect snow are frozen, while those turned downward may not be injured. WEST and EDLEFSEN[3] attempted to prevent frost injury to apricot buds by spraying the trees with water. Instead of the desired effect of preventing freezing, this procedure evidently killed the tissue by allowing inoculation from ice formed on the surface. Trees which were not sprayed with water were not injured, although subjected to the same temperature. These examples serve to illustrate the importance of surface inoculation in producing frost injury.

The amount of undercooling in plants is not generally very great, nor is it sufficient to account for true frost hardiness. Such herbaceous plants as cabbage, kale, turnips, however, which ordinarily can withstand a considerable degree of freezing, acquire hardiness quite rapidly. In the case of cabbage, a temperature of 3°C. was found to harden the plants sufficiently in 5 days to allow them to be frozen stiff at −3°C. without injury. The principal importance, therefore, of the epidermal coverings for the frost resistance of such plants appears to be that they allow the plants which possess them to withstand temperatures somewhat below zero until the cells are able to adapt themselves physiologically to the changes incident upon freezing.

Summary

Undercooling of the tissues occurs to a greater degree in such herbaceous plants as possess protective epidermal coverings than in plants not so protected. The undercooling in such plants is not due to substances in the cell sap, but mainly to the prevention of inoculation from ice formed on the surface of the tissue. A method is given for determining electrically the temperatures within leaf tissues.—R. B. HARVEY, *Bureau of Plant Industry, Department of Agriculture, Washington, D.C.*

[3] WEST, F. S., and EDLEFSEN, N. E., Orchard heating. Bull. 161, Utah Agric. Coll. Exp. Sta. 1917.

CURRENT LITERATURE

NOTES FOR STUDENTS

Conditions affecting flower development.—KLEBS[1] divides the process of flower formation by the rosettes of *Sempervivum Funkii* and *S. albidum* into 3 distinct, successive steps: (1) production of the condition of ripeness to flower (*blühreife Zustand*), (2) formation of flower primordia, and (3) development of flower clusters and elongation of the axis. Light is the dominant factor in determining all 3 of these stages of development.

In the first and third, light is effective entirely through its photosynthetic action, and its effectiveness rises with its energy value. Higher temperatures counteract light by favoring dissimilation. Accordingly, the effect of temperatures can in part be annulled by increased light intensities. It is the balance of assimilation over dissimilation that furthers the development of these 2 stages. KLEBS finds that at lower temperatures (about 6°C.) both these stages can be attained in darkness, although in the last it gives a far less extensive inflorescence. He thinks this is likewise tied up with a balance in favor of available carbon synthate. The low temperature gives low respiration and leads to the accumulation of soluble sugars by the hydrolysis of insoluble carbohydrates.

In the second step, formation of flower primordia, light has 2 distinct and antagonistic effects. The one which favors the process is due to the photosynthetic activity of the light and is a function of the less refrangible rays of the spectrum. The other, which inhibits the process or even annuls the ripe to flower condition, must at present be termed a stimulus effect, and it is a function of the less refrangible blue rays. Diffuse daylight is relatively injurious to primordia development because of the high percentage of blue violet rays it contains. The Osram light and direct sunlight favor this development because of the dominance of the red rays.

KLEBS says it is still an unanswered question whether inflorescence development in other forms and in plants in general can be divided into these 3 distinct steps with similar light effects in each step. He suggests some facts as evidence that such may be the case. His past work has done much to show that the formative effects of conditions on plants is largely through the nutrient effects of these conditions. Thus the formative effect of light is explained in a large part by its effect on carbon assimilation, but KLEBS points out here, as in his

[1] KLEBS, GEORGE, Über die Blütenbildung von *Sempervivum*. Festschrift zum ERNST STAHL. pp. 128–151. Jena. 1918.

older work, that there is also a specific formative action of the blue rays as yet unexplainable on the nutrient basis.

He has often distinguished between the amount of carbon synthate and the amount of salt nutrients as formative factors in the plant, especially in connection with reproduction; and now FISCHER[2] makes this more definite by considering the nitrogen supply as the most important formative factor furnished by the salts, and by speaking of the carbon nitrogen ratio (C/N) of plants. He probably would not deny that the supply of other nutrient elements, phosphorus, calcium, potassium, etc., have at least minor formative effects and often of an opposite nature from nitrogen. This ratio can be increased by increasing the photosynthesis of the plants or by decreasing the nitrogen supply. The ratio can be decreased by decreasing photosynthesis or by increasing the nitrogen supply. FISCHER comes to this important conclusion. Very high C/N in plants favor flowering, while a low C/N favors vegetation. His conclusions are largely based on his own work on the effect of increased partial pressure of carbon dioxide upon the development of plants, but not upon chemical analysis of the tissues.

KRAUS and KRAYBILL[3] have recently worked upon the tomato, varying the C/N in it by varying its nitrogen supply. On the basis of extensive cultures and chemical, microchemical, and anatomical studies, they come to the following conclusions: (1) a very high C/N gives little vegetative growth and poor reproduction with a high percentage of dry matter; (2) medium C/N gives moderate vegetation growth, good reproduction, and a medium percentage of dry matter; (3) very low C/N gives very vigorous vegetative growth, little reproduction, and a low percentage of dry matter. KRAUS'S extensive horticultural investigations enable him to give much evidence that the C/N ratio is a factor of great significance in determining fruitfulness in many economic plants. The contribution apparently puts into the hands of producers one of the important means of controlling fruitfulness. FISCHER'S less extensive and one-sided attack caused him to miss the fact that a very high C/N not only reduces vegetative growth but diminishes reproduction.

These papers have thrown much light on some of the nutrient factors modifying vegetation and reproduction in plants.—WM. CROCKER.

Loss of chlorophyll.—MEYER[4] notes that in *Tropaeolum majus*, growing in pots in a greenhouse, the young leaves at the top of the stem are dark green, while the progressively older ones down the stem are green, bright green, yellow

---

[2] FISCHER, H., Zur Frage der Kohlensaure-Einahrung der Pflanzen. Gartenflora **65**: 232–237. 1916.

[3] KRAUS, E. J., and KRAYBILL H. R., Vegetation and reproduction with special reference to the tomato. Oreg. Agric. Exper. Sta. Bull. 149. pp. 90. 1918.

[4] MEYER, ARTHUR, Eiweiszstoffwechsel und Vergilben der Laubblätter von *Tropaeolum majus*. Festschrift zum ERNST STAHL. pp. 85–127. Jena. 1918.

green, yellow and bright yellow, and finally the oldest ones on the plant are wilting. MEYER points out that this change in color is due to the gradual decomposition of the chlorophylls, while the carotin and xanthophyll remain constant. As this change progresses the chloroplasts become smaller, and in later stages are shriveled granular masses with balls of excreted material about them. With the gradual loss of chlorophyll goes a similar decomposition of the proteins of the chloroplast. It should be mentioned that MEYER adduces evidence for the view that the chloroplast is the main organ for the storage of the proteins manufactured in the foliage leaf, if indeed not the very seat of protein manufacture. The amount of carbohydrates in the leaves also falls with age. MEYER found that when leaves are placed in darkness no reduction occurs in the proteins until the carbohydrates are greatly reduced by respiration. The decomposition of the proteins then begins, he believes, as a source of carbon chains for respiration. He states that there is no loss of nitrogen from the leaf during this change, but that the nitrogen residue remains in the leaf, while the carbon chain of the protein is used for respiration. He apparently gives the following interpretation of the process: As the leaves become older they become weakened; in this weakened condition the photosynthetic power falls; this leads to a great reduction in the amount of carbohydrates in the leaf, and finally to the decomposition of the proteins of the chloroplasts as a carbon source for respiration; this decomposition of the proteins is accompanied by the decomposition of the chlorophyll and the change in color.

SCHERTZ, in an unpublished work from this laboratory, finds in many respects parallel behavior in *Coleus Blumei*. He finds that shortage of nitrates leads to the decomposition of the chlorophyll, and that old leaves can be maintained green by addition of nitrogen fertilizer. He also finds the phospholipine content of the leaf greatly reduced as yellowing progresses. His evidence seems good that shortage of nitrogen initiates all of the decomposition of nitrogen compounds (chlorophyll, phospholipines, and proteins), and that it must be looked at as the immediate cause of the loss of chlorophyll. Plants grown in pots are likely to become pot bound and limited in their supply of soil nutrients.

There are many incompletely worked phases in MEYER'S paper; he has filled in some gaps by drawing data from other workers on very different materials; and his work leaves much to be desired in quantitative determinations and cultural experiments. All these leave interpretation to bridge broad chasms, and it is therefore not strange if he has missed the initiating cause of loss of chlorophyll.

If SCHERTZ is right, that the decomposition of chlorophyll in *Coleus Blumei* is due to shortage of nitrogen as a building material, it is also conceivable that a great excess of nitrogen may sometimes lead to the decomposition of chlorophyll due to the dearth of carbon chains produced by the excess of nitrogen. Shortage of magnesium as a building material may sometimes act in a similar way.—WM. CROCKER.

Knop's solution.—TOOLE and TOTTINGHAM[5] find that additions of $Fe(OH)_3$ to Knop's solution greatly increases the growth of barley tops in it (21 day cultures), while additions of carbon black depress the growth of tops and additions of H_2SiO_3 have no effect. None of these additions affect the growth of the roots. Part of the beneficial action of the $Fe(OH)_3$ may be due to its neutralizing action on the acids of the solution. It is interesting to note that the higher additions of $Fe(OH)_3$ removed 90 per cent of the phosphorus from solution.

In another piece of work, TOTTINGHAM[6] has shown that he can displace more than 90 per cent of the $MgSO_4$ of Knop's solution with $Mg(NO_3)_2$ without interfering with the growth of red clover, a rather heavy sulphur requiring plant. It is evident that some of the nutrients in the commonly used nutrient solutions are far beyond the minimum concentration necessary to give the plant its optimum supply, and that the so-called optimum concentration of the solution is determined by other factors than optimum supply. The conditions of and the mechanism for absorption from the soil (root hairs with their acid pectic layer in contact with soil particles bearing certain nutrients in compounds of low solubility) are quite different. Work with water cultures has established some very fundamental principles in soil fertility (essential nutrient elements, necessity of balanced solutions, etc.). It is a question how much more this method alone is capable of adding to our knowledge of soil fertility. In the present concentrated nutrient solutions with which we are working we may be mainly playing the toxic concentration of one salt against the toxic concentration of another in a way to get the least possible injury.—WM. CROCKER.

Chromosomes in Carex.—Oogenesis and spermatogenesis have been studied by HEILBORN[7] in several species of *Carex*, special attention being given to chromosome numbers, which vary greatly in this genus. The gametophyte numbers in the forms investigated are as follows: *Carex pilulifera* 8, *C. ericetorum* 16, *C. digitata* 24, *C. caryophylla* and *C. flava* 32. JUEL had already reported 52 for *C. acuta*, and STOUT 37 for *C. aquatilis*. It is interesting to note that *C. pilulifera* has the largest chromosomes, and that in species with higher numbers the chromosomes are correspondingly smaller. Attempts to cross the various species have not yet proved successful, but the work is still in progress.—C. J. CHAMBERLAIN.

---

[5] TOOLE E. H., and TOTTINGHAM, W. E., The influence of certain added solids upon the composition and efficiency of Knop's nutrient solution. Amer. Jour. Bot. **5**:452–461. 1918.

[6] TOTTINGHAM, W. E., Sulfur requirement of red clover plant. Jour. Biol. Chem. **36**:429–438. 1918.

[7] HEILBORN, OTTO, Zur Embryologie und Zytologie einiger *Carex*-Arten. Svensk Botanisk Tidskrift **12**:212–220. *figs. 1–14.* 1918.